Journal of Chemical Crystallography, Vol. 29, No. 4, 1999

The structure and conformation of the tryptophanyl diketopiperazines cyclo(Trp–Trp)·C_2H_6SO and cyclo(Trp–Pro)

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Received August 6, 1998

The structure and conformation of the cyclic dipeptides [cyclo(L-Trp–L-Trp)·C_2H_6SO] and cyclo(L-Trp–L-Pro) have been investigated with X-ray crystallographic and spectroscopic methods. Cyclo(L-tryptophanyl-L-tryptophanyl)·DMSO solvate crystallized in the space group $P2_12_12_1$ with cell dimensions a = 6.193(2), b = 11.545(3), c = 31.117(4) Å. The crystal structure is stabilized by four hydrogen bonds (three intermolecular hydrogen bonds and one intramolecular bond). The first intermolecular bond is between the oxygen of DMSO and the nitrogen of indole ring 2, in contrast to the second intramolecular hydrogen bond between the nitrogen of indole ring 1 and the oxygen of DMSO. The two remaining intermolecular hydrogen bonds are between the nitrogens of the DKP ring and the carbonyl oxygens of the DKP ring. The values of χ_1^{1A} (−45.764) and χ_2^{1A} (67.437) indicate an extended side chain conformation for Trp residue 1 (E_N) and a folded conformation for Trp residue 2. The DKP ring is more planar than in other cyclic dipeptide compounds (φ_1 = 11.414, Ψ_1 = −7.516, φ_2 = 12.471, and Ψ_2 = −8.256). In cyclo(L-Trp–L-Trp) the Cβ resonance of L-tryptophan (29.88 ppm) is shifted upfield 0.82 ppm when compared with the same resonance in cyclo(L-Trp–L-Gly) (30.7 ppm) and cyclo(L-Leu–L-Trp) (30.7 ppm). Two conformations of cyclo(Trp–Pro) crystallized in the space group P1 with cell dimensions a = 5.422(1), b = 9.902(1), c = 13.443(2) Å, α = 80.42(1), β = 78.61(1), and γ = 89.13(1)°. The conformation of the backbone and the orientation of the aromatic side chains for these conformers are very similar. The DKP rings for both conformers adopt a typical boat conformation in contrast to the flattened chair conformation observed for cyclo(Tyr–Pro) and cyclo(Phe–F-Pro). The tryptophan side chains of these conformers are folded towards the diketopiperazine (DKP) ring. The pyrrolidine ring for conformer 1 can be described as an envelope (Cs–Cβ-*endo*) conformation in contrast to the pyrrolidine ring symmetry for conformer 2 which is an intermediate between C_s and C_2 rather than pure C_s for the proline ring with Cβ-*endo* and Cγ-*exo* with respect to C′. The two prolyl rings are puckered at the β-carbon atoms which deviate from the best planes defined by the four remaining atoms. The crystal structures are stabilized by four intermolecular hydrogens bonds. An intermolecular bond between the nitrogen of the indole ring (conformer 1) and the carbonyl oxygen of the DKP ring (conformer 2) was observed. The second hydrogen bond is between the nitrogen of the indole ring (conformer 2) and the carbonyl oxygen of the DKP ring (conformer 1). The last two hydrogens involve the carbonyl oxygens of the DKP rings and the nitrogens of the DKP rings [carbonyl oxygen of DKP ring (conformer 1)––––nitrogen of DKP ring (conformer 2); nitrogen of DKP ring (conformer 1)––––––carbonyl oxygen of DKP ring (conformer 2)].

KEY WORDS: Cyclic dipeptides; Tryptophan; Proline; x-rays, NMR.

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Introduction

Cyclic dipeptides, both in the solid state and in solution, are extensively used as suitable models to gain information on more complex peptides and proteins. Due to their relative simplicity 2,5-diketopiperazines (DKP's) are excellent models for theoretical studies and are of primary interest for comparison with experimental findings obtained through X-ray experiments and NMR studies. The properties of DKP's differ from those of ordinary peptides:

* They do not exist as zwitterions and are often neutral compounds.[1]
* The simpler members of this group are water soluble.[1]
* They provoke the destruction of the secondary globular protein structure.[2]
* They are sensitive to oxidation, especially when *imino* acid residues are the building blocks.[3]
* They may act as powerful hydrolytic catalysts.[4]
* They are of interest in studies on the thermodynamic behavior of non-ionic compounds in aqueous medium because they have the ability of forming hydrogen bonds with the solvent (via the two *cis*-amide groups in the ring) and of giving rise to hydrophobic interactions (determined by the R substituents).[5]
* Many derivatives show antiviral properties while others are powerful antibiotics, and anti-tumor agents.[6,7,8]

The inclusion/incorporation of essential aromatic amino acids offers a model system of limited complexity for studying the influence of solvents and solvent mixtures on intramolecular interactions during the excited lifetime of the chromophore.[9] Tryptophan contains an indole ring (a benzene ring fused to a pyrrole ring) which is found in many pharmacologically active compounds, including hallucinogens and other drugs which have mental or emotional effects, e.g., LSD (Lysergic acid diethylamide), Psilocybin, DMT (Dimethyltryptamine), Harmaline, and Strychnine.

The therapeutic uses of drugs containing an indole ring that are available on the market today range from anti-emetics, and anti-inflammatories to the treatment of hypertension, migraine, and Parkinson's disease (e.g., Bromocriptine, Ergotamine, Indapamide, Indomethacin, Sumatriptan, and Ondansetron).

Proline is an important *imino* acid of many proteins and neuropeptides and imposes certain conformational restraints on these biomolecules.[10,11] In addition, the conformational aspects of the pyrrolidine ring system are of particular interest as they reveal different modes of puckering of the five-membered ring system.[12] Second, proline is the only residue which leads to an N-alkylamide bond when incorporated into a peptide via natural biochemical pathways. Numerous peptides with important biological activity (e.g., didemnin and cyclosporin) contain N-methyl amino acids.[13] Thirdly, the *cis–trans* isomerism of the N-alkylamide bond involving the amino group of proline has been implicated in the biological activity of peptides. Brandl and Deber[14] have proposed that *cis–trans* isomerism of proline residues might play a role in transduction of transmembrane proteins.

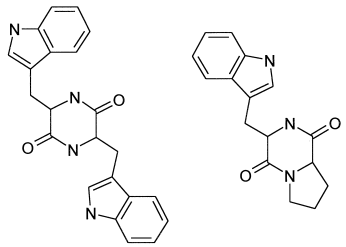
In continuation of our interest in conformationally restricted small peptides containing the prolyl residue and/or the aromatic amino acid residue, and as part of our studies on the biological activity of selected cyclic dipeptides, we report here the synthesis, conformational and spectroscopic properties of cyclo(L-Trp–L-Trp) and cyclo(L-Trp–L-Pro) (Scheme 1).

Experimental

All reagents and solvents were of reagent grade and used without further purification.

Synthesis of cyclo(Trp–Trp)

Triethylamine (0.96 ml, 6.89 mmol) and diethylphosphoryl cyanide (0.52 ml, 3.61 mmol) were added



Scheme 1.

Table 1. Crystal Data and Summary of Intensity Data Collection and Structure Refinement of cyclo(Trp–Trp) and cyclo(Trp–Pro)

Compound	$C_{22}H_{20}O_2N_4{\cdot}C_2H_6SO$	$C_{16}H_{17}O_2N_3$
CCDC deposit no.	CCDC-1003/5497	CCDC-1003/5498
Color/shape	Colorless/parallelepiped	Colorless/parallelepiped
Formula weight	450.55	283.33
Space group	$P2_12_12_1$	$P1$
Temp., °C	18	18
Cell constants[a]		
a, Å	6.193(2)	5.422(1)
b, Å	11.545(3)	9.902(1)
c, Å	31.117(4)	13.443(2)
β, deg		78.61(1)
Cell volume, Å^3	2224.9	697.5
Formula units/unit cell	4	2
D_{calc}, g cm^{-3}	1.34	1.35
μ_{calc}, cm^{-1}	1.38	0.53
Max. crystal dim., mm	0.40 × 0.08 × 0.13	0.57 × 0.15 × 0.30
Reflections measured	2819	4444
2θ range, deg	$3 \leq \theta \leq 27$	$3 \leq \theta \leq 30$
Range of h, k, l	−7, −14, −39	0.7, −13.13, −18.18
Reflections observed		
[$>2\sigma(1)$][b]	1268	2786
Diffractometer/scan	Enraf-Nonius CAD-4/ω-2θ	Enraf-Nonius CAD-4/ω-2θ
Radiation, graphite monochromator	MoKα(λ = 0.71073)	Mo$K\alpha$(λ = 0.71073)
Computer programs[c]	SHELX76	SHELX76
Structure solution	SHELX86, SHELX76	SHELX86, SHELX76
No. of parameters varied	224	377
Weights	$\sigma^{-2}(F_{obs})$	$\sigma^{-2}(F_{obs})$
$R = \Sigma\|\|F_o\| - \|F_c\|\|\Sigma\|F_o\|$	0.1199	0.0739
R_w	0.0544	0.0386
Largest feature final diff. map	1.11e$^-$ Å^{-3}	0.410e$^-$ Å^{-3}
	−1.14e$^-$ Å^{-3}	−0.383e$^-$ Å^{-3}

[a] Least-squares refinement of $[(\sin\theta)/\lambda]^2$ values for 25 reflections $\theta > 20$ deg.
[b] Corrections: Lorentz-polarization.
[c] Neutral scattering factors and anomalous dispersion corrections.
* Ellipsoids were at a 50% level.

to a stirred solution of N-t-Boc–L-Trp (1 g, 3.28 mmol) and L-Trp–OMe (0.84 g, 3.28 mmol) in 1,2-dimethoxyethane (40 ml) at 0°C. After 1 h at 0°C and 4 h at room temperature the reaction mixture was diluted with chloroform (250 ml) and washed successively with 5% hydrochloric acid (50 ml), aqueous sodium hydrogen carbonate (50 ml), and saturated brine (50 ml). Removal of the solvent *in vacuo* furnished the protected product as a colorless syrup [Rf = 0.66, chloroform–methanol–acetic acid (14 : 2 : 1)].

The N–t-Boc dipeptide ester was dissolved in formic acid (20 ml, 98%) containing anisole (0.2 ml) and stirred for 3 h at room temperature. After removal of the excess formic acid *in vacuo*, the residue containing the crude dipeptide ester formate was refluxed in 40 ml of *sec*-butanol and toluene (4 : 1) for 3 h at 120°C. After concentrating the solution to 8 ml and cooling to 0°C, the product was filtered off and recrystallized from a suitable solvent. [yield 70%; Rf = 0.55, chloroform–methanol–acetic acid (14 : 2 : 1)].

Synthesis of cyclo(Trp–Pro)

Triethylamine (0.96 ml, 6.89 mmol) and diethylphosphoryl cyanide (0.55 ml, 3.61 mmol) were added to a stirred solution of N–t-Boc–L-Trp (1 g, 3.28 mmol) and L–Pro–NH_2 (0.37 g, 3.28 mmol) in 1,2-dimethoxyethane (40 ml) at 0°C. After 1 h at 0°C and 4 h at room temperature, the reaction mixture

Table 2. Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Thermal Factors ($Å^2 \times 10^3$) for cyclo (Trp–Trp)·DMSO

Atom	*x/a*	*y/b*	*z/c*	U_{eq}[a]
N(1)	5415(17)	3(8)	−2150(3)	28(3)
C(1)	7224(22)	794(11)	−2134(4)	26(4)[b]
C(2)	9409(23)	272(12)	−2245(5)	40(4)
O(1)	10953(17)	925(9)	−2272(4)	60(3)
N(2)	9436(20)	−881(9)	−2295(3)	31(3)[b]
C(3)	7726(21)	−1703(11)	−2232(5)	31(3)
C(4)	5468(23)	−1160(12)	−2184(5)	29(3)
O(2)	3925(15)	−1799(8)	−2187(3)	41(3)
C(5)	7393(23)	1338(12)	−1677(4)	42(4)
C(6)	5361(25)	1755(12)	−1491(5)	41(4)
C(7)	4213(25)	1163(12)	−1212(5)	44(4)
N(3)	2352(22)	1777(11)	−1078(4)	43(4)[b]
C(8)	2480(26)	2847(11)	−1269(5)	38(4)[b]
C(9)	1173(25)	3806(12)	−1233(5)	48(4)[b]
C(10)	1692(27)	4763(12)	−1480(4)	57(5)[b]
C(11)	3521(27)	4819(13)	−1742(5)	58(5)[b]
C(12)	4802(24)	3895(12)	−1767(4)	39(4)[b]
C(13)	4341(24)	2884(11)	−1520(4)	31(4)[b]
C(14)	8241(21)	−2513(11)	−1851(4)	35(3)
C(15)	8253(26)	−1934(12)	−1430(5)	38(4)[b]
C(16)	9854(24)	−1229(11)	−1273(5)	46(4)
N(4)	9318(26)	−849(11)	−853(4)	68(4)
C(17)	7363(32)	−1287(14)	−738(6)	55(5)[b]
C(18)	6274(27)	−1174(13)	−364(5)	58(5)[b]
C(19)	4320(29)	−1763(12)	−336(5)	60(5)[b]
C(20)	3602(31)	−2438(13)	−664(5)	70(6)[b]
C(21)	4652(25)	−2549(12)	−1047(5)	47(5)[b]
C(22)	6727(25)	−1962(12)	−1106(5)	42(4)
O(3)	826(19)	899(9)	−249(3)	65(3)
S(1)	1888(15)	1014(5)	172(2)	132(3)
C(23)	1463(30)	2314(12)	408(5)	83(5)
C(24)	971(33)	66(12)	543(5)	110(6)

[a] $U_{eq} = 1/3\ \Sigma_i\ \Sigma_j\ U_{ij} a_i^* a_j^* (\mathbf{a}_i \cdot \mathbf{a}_j)$.
[b] Isotropic temperature factor.

was diluted with ethylacetate (250 ml) and washed successively with 5% hydrochloric acid (50 ml), aqueous sodium hydrogen carbonate (50 ml), and saturated brine (50 ml). Removal of the solvent *in vacuo* furnished the protected product as a colorless syrup [Rf = 0.68, chloroform–methanol–acetic acid (14:2:1)].

Colorless crystals of the protected dipeptide crystallized from ethylacetate-n-hexane

The N–t-Boc–dipeptide (0.2 g, 1.24 mmol) was dissolved in formic acid (20 ml, 98%) containing anisole (0.2 ml) and stirred for 3 h at room temperature. After removal of the excess formic acid *in vacuo,* saturated $NaHCO_3$ (50 ml) was added to the above crude dipeptide formate salt and the mixture was stirred for 3 days at low temperature (5–8°C). The cyclic dipeptide cyclo(Trp–Pro) was extracted with chloroform (4 × 30 ml) and crystals were grown from chloroform-n-hexane [Rf = 0.58, chloroform–methanol–acetic acid (14:2:1); Rf = 0.19, isopropyl ether–chloroform–acetic acid (6:3:1)].

X-ray analysis

All diffraction measurements were obtained at room temperature and the data was collected with an Enraf Nonius CAD4 diffractometer with MoK_α radiation (Graphite monochromator, λ = 0.7107 Å). Accurate unit cell parameters were obtained by least-squares methods from the position of 25 centered reflections for each crystal. There was no significant crystal decay and intensities were corrected for absorbtion, as well as Lorentz and polarization effects. An empirical method for absorption correction was applied.[15] Standard intensity checks and orientation control were carried out. The structures were solved by Patterson and direct methods.[16,17] Refinement was by full matrix least-squares methods, using σ^{-2} (F_{obs}) weights.[17] All the nonhydrogen atoms for cyclo(Trp–Trp) and cyclo(Trp–Pro) were refined anisotropically. Atomic scattering factors were taken from the literature.[18]

Diffraction quality crystals of cyclo(Trp–Trp) were obtained by slow cooling of a hot DMSO solution. The crystals crystallized from DMSO in the space group $P2_12_12_1$ and are listed with other relevant crystal data in Table 1. Due to the low ratio of number of reflections: number of parameters, a selection of atoms were refined anisotropically. All hydrogen atoms, except the experimentally located and refined HN3, were placed in calculated positions and were included in the refinement with common isotropic thermal parameters. Fractional coordinates and equivalent thermal factors, and the relevant torsion angles for cyclo(Trp–Trp) are listed in Table 2 and Table 3, respectively.

Diffraction quality crystals of cyclo(Trp–Pro) crystallized from chloroform-n-hexane in the space group $P1$ and are listed with other relevant data in Table 1. All hydrogen atoms were placed in calculated positions and were included in the refinement with common isotropic thermal parameters.

Perspective views of the molecules, prepared with ORTEP,[19] are represented in Figs. 1 and 2, illustrating the crystallographic numbering schemes used.

Table 3. The Relevant Torsion Angles of cyclo(Trp–Trp)·DMSO

Torsion angle					
C4	N1	C1	C2	φ_1	11.414(1.881)
C2	N2	C3	C4	φ_2	12.471(1.931)
N1	C1	C2	N2	Ψ_1	−7.516(1.869)
N2	C3	C4	N1	Ψ_2	−8.256(1.806)
C1	C2	N2	C3	ω_1	−4.259(2.278)
C1	N1	C4	C3	ω_2	−3.107(1.911)
				Indole residue 1 or (Trp side chain 1)	Indole residue 2 or (Trp side chain 2)
N1(N2)[a]	C1(C3)	C5(C14)	C6(C15)	−45.764(1.702)	67.437(1.612)
C2(C4)	C1(C3)	C5(C14)	C6(C15)	−172.464(1.275)	−60.795(1.649)
C1(C3)	C5(C14)	C6(C15)	C13(C22)	−89.748(1.926)	104.126(1.865)
C1(C3)	C5(C14)	C6(C15)	C7(C16)	99.018(1.763)	−75.895(1.999)
C5(C14)	C6(C15)	C7(C16)	N3(N4)	178.669(1.337)	−178.897(1.391)
C13(C22)	C6(C15)	C7(C16)	N3(N4)	5.628(1.891)	1.086(1.908)
C5(C14)	C6(C15)	C13(C22)	C8(C17)	−177.138(1.412)	179.144(1.542)
C7(C16)	C6(C15)	C13(C22)	C8(C17)	−4.708(1.752)	−0.839(2.075)
C6(C15)	C7(C16)	N3(N4)	C8(C17)	−4.468(1.732)	−0.992(1.947)
N3(N4)	C8(C17)	C13(C22)	C6(C15)	2.056(1.724)	0.258(2.132)
C9(C18)	C8(C17)	C13(C22)	C12(C21)	−4.682(2.201)	0.963(2.411)
C11(C20)	C12(C21)	C13(C22)	C8(C17)	3.163(2.185)	−2.018(2.195)
C7(C16)	N3(N4)	C8(C17)	C13(C22)	1.194(1.737)	0.430(1.849)
C13(C22)	C8(C17)	C9(C18)	C10(C19)	4.761(2.205)	−0.855(2.582)
C10(C19)	C11(C20)	C12(C21)	C13(C22)	−1.820(2.343)	3.248(2.568)
C8(C17)	C9(C18)	C10(C19)	C11(C20)	−3.502(2.430)	1.823(2.588)
C9(C18)	C10(C19)	C11(C20)	C12(C21)	2.068(2.392)	−3.174(2.766)

[a] Atom designation of Trp residue 1 with Trp residue 2 in brackets.

Fractional coordinates and equivalent thermal factors, and the relevant torsion angles for cyclo(Trp–Pro) are listed in Table 4 and Table 5, respectively.

Spectroscopic analyses

^{1}H Proton (300 MHz) and ^{13}C carbon (75 MHz) spectra were recorded on a Bruker AM-300 spectrometer, with DMSO-d_6, and $CDCl_3$ as solvents, and TMS as internal standard. Hetcor and Cosy spectra were recorded to assist with the ^{1}H and ^{13}C assignments.

Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer as KBr disks. Fast atom bombardment (FAB) mass spectra of both cyclo(Trp–Trp) and cyclo(Trp–Pro) dissolved in DMSO with 3-nitrobenzyl alcohol as matrix, were obtained on a VG-7070E spectrometer.

Results and discussion

X-ray analysis

Figures 1 and 2 show the ORTEP drawing of cyclo(Trp–Trp)·DMSO and cyclo(Trp–Pro) complete with their respective numbering schemes. The X-ray data appears in Table 1 and the torsion angles of the backbone and the relevant side chains appear in Tables 3 and 5.

The X-ray data (Table 1) indicated the crystallization of only one conformation for cyclo(Trp–Trp) in comparison with the two conformations for cyclo(Trp–Pro). The DKP ring is appreciably more planar than in other cyclic dipeptide compounds due to the conformational angles being reduced to φ_1 = 11.414, Ψ_1 = −7.516, φ_2 = 12.471, and Ψ_2 = −8.256. The values of χ_1^{1A} (−45.764) and χ_2^{1A} (67.437) indicate an extended side chain conformation for Trp residue 1 (E_N) and a folded conformation for Trp residue 2. The extended side chain conformation for indole ring

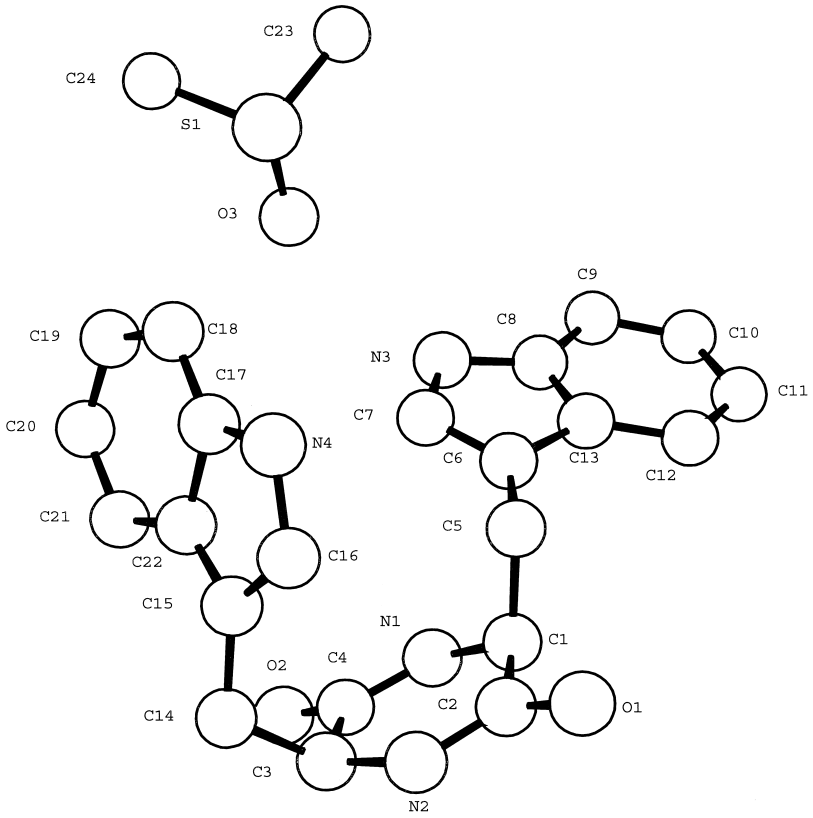


Fig. 1. ORTEP view of cyclo(Trp–Trp) · DMSO.

1 is in agreement with Morris *et al.*,[20] who observed an extended conformation of the tryptophan side chain in crystals of cyclo(Trp–Gly). However, a folded conformation is characteristic of some cyclic dipeptides containing an aromatic side chain.[21–25] The amide bonds ω_1 and ω_2 are −4.259 and −3.107, respectively (Table 3), and deviate only slightly from planarity. Such angles have been found to be as large as 8° in cyclic dipeptides.[26] The internal angles at Cα in cyclo(Trp–Trp) are 114.5 and 115.6° whereas in unsubstituted diketopiperazines the corresponding angles are 115.1°.[27] The φ (11.414, 12.471) and Ψ (−7.516, −8.256) values are smaller and of opposite sign to the corresponding angles in cyclo(L-Trp–L-Pro): conformer 1 [(φ = −43.739, −38.954) (Ψ = 39.005, 34.682)]; conformer 2 [(φ = −43.272, −38.056) (Ψ = 40.431, 35.415)].

The crystal packing is stabilized by four hydrogen bonds: N4—HN4—O3, inter; N3—HN3—O3 intra; N1—HN1—O1, inter; N2—HN2—O2, inter. Two of the hydrogen bonds involve the nitrogens of the two indole rings and the oxygen of DMSO. The two remaining hydrogen bonds are between the nitrogens of the DKP ring and the carbonyl oxygens of the DKP ring (Table 6). *Molecular modeling* of cyclo(Trp-Trp) is given in Fig. 3.

The X-ray data (Table 1) indicated the crystallization of two conformations for cyclo(Trp–Pro) as shown in Fig. 2. The two conformers show general similarity with respect to conformational orientation for the backbone around the following torsion angles (conformer 1: φ_1 = −43.739, φ_2 = −38.954, Ψ_1 = 39.005, Ψ_2 = 34.682; conformer 2: φ_1 = −43.272, φ_2 = −38.056, Ψ_1 = 40.431, Ψ_2 = 35.415) and the orientation of the tryptophan side chains (conformer 1: χ_1^{1A} = 62.027, χ_1^{2A} = 167.964; conformer 2: χ_1^{1A} = 60.934, χ_1^{2A} = 171.214). The DKP ring of cyclo(Trp–Pro) for both conformers can be considered a typical boat conformation with the Cα—Cβ bond of Pro orientated equatorially and is in agreement with the observation that the majority of DKP's prefer a deep *boat-like* conformation for the DKP ring with the Cα—Cβ bond of Pro orientated equatorially.[28,29] This is in contrast to the conformation of the DKP ring of cyclo(Tyr–Pro)[30] and cyclo(Phe–F-Pro)[31], that can be considered a *flattened chair* with the Cα—Cβ bond

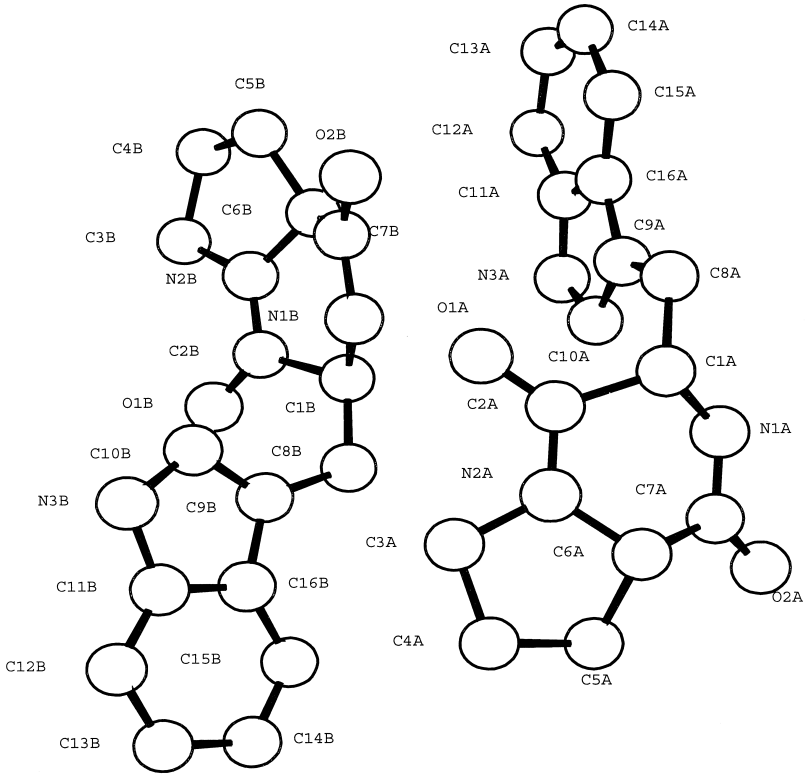


Fig. 2. ORTEP view of cyclo(Trp–Pro).

of Pro and the Cα—Cβ bond of Tyr orientated equatorially and axially, respectively. The orientations of the aromatic ring for both the conformers are folded towards the DKP ring. These side chain conformations are very similar, although not identical to the folded arrangement of cyclo(Tyr-Pro) [χ_1^1 = 64.1 (conformer 1); χ_1^1 = 64.2, (conformer 2)][30] and cyclo(Phe—F-Pro) (χ_1^1 = 65.7)[31] and in contrast to the extended N, N_E for the Phe side chain of cyclo(Phe—Pro) (χ_1^1 = −79.7).[32]

The small values for the conformational angle χ_2^4 [C$^\gamma$—C$^\delta$-N—C$^\alpha$], −1.086 (conformer 1) and −3.009 (conformer 2) indicate that the two prolyl rings are puckered at the β-carbon atoms which deviate from the planes defined by the four remaining atoms.[33] The conformation of the pyrrolidine ring of cyclo(Trp–Pro) conformer 1 can be described as an envelope (C_s—Cβ-*endo*) conformation[34] and the puckering mode for conformer 2 as an intermediate[34] between C_s and C_2 with Cβ-*endo* and Cγ-*exo* with respect to C′.

The amide bonds ω_1 and ω_2 are 0.230°; 5.649° (conformer 1) and −1.017°; 4.816° (conformer 2), respectively. In cyclo(Trp–Pro), the internal angles at Cα follow an expected trend [110.4°, 108.6° (conformer 1) and 110.4°, 108.8° (conformer 2)], and are smaller than the corresponding angles (115.1°) in the unsubstituted diketopiperazine cyclo(Gly–Gly).[27]

The crystal packing is stabilized by four intermolecular hydrogen bonds: N3A—HN3A—O2B, N3B—HN3B—O2A, N1A—HN1A—O1B, and N1B—HN1B—O1A. The first hydrogen bond is between the nitrogen of the indole ring (conformer 1) and the carbonyl oxygen of the DKP ring (conformer 2). The second hydrogen bond is between the nitrogen of the indole ring (conformer 2) and the carbonyl oxygen of the DKP ring (conformer 1). The two remaining hydrogens involve the carbonyl oxygens of the DKP rings and the nitrogens of the DKP rings [carbonyl oxygen of DKP ring (conformer 1)–––– nitrogen of DKP ring (conformer 2), nitrogen of DKP ring (conformer 1)–––––carbonyl oxygen of DKP ring (conformer 2)] (Table 7).

Spectroscopic analyses

The mass spectra of cyclo(Trp–Trp) and cyclo(Trp–Pro) show a parent ion peak at *m/z* 373

Table 4. Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Thermal Factors ($Å^2 \times 10^3$) for cyclo(Trp–Pro)

Atom	x/a	y/b	z/c	U_{eq}[a]
N(1A)	7242	−4255	7867	41(1)
C(1A)	4629(13)	−3868(7)	8285(7)	41(2)
C(2A)	4263(15)	−2359(7)	7838(6)	37(2)
O(1A)	2961(13)	−1602(6)	8343(5)	51(1)
N(2A)	5366(14)	−2019(6)	6862(6)	45(1)
C(3A)	5040(18)	−693(8)	6246(7)	57(2)
C(4A)	6425(19)	−834(9)	5174(7)	75(3)
C(5A)	8276(17)	−2020(8)	5340(7)	66(2)
C(6A)	6806(15)	−2972(8)	6245(6)	43(2)
C(7A)	8337(16)	−3909(8)	6884(6)	44(2)
O(2A)	10502(13)	−4262(6)	6506(5)	60(1)
C(8A)	3980(15)	−4184(7)	9449(6)	40(2)
C(9A)	5492(15)	−3459(8)	10021(7)	39(2)
C(10A)	7788(15)	−2749(7)	9646(7)	40(2)
N(3A)	8561(14)	−2290(6)	10457(6)	44(1)
C(11A)	6784(16)	−2678(7)	11336(7)	38(2)
C(12A)	6729(17)	−2416(7)	12326(7)	50(2)
C(13A)	4690(19)	−2949(8)	13096(7)	58(2)
C(14A)	2716(17)	−3699(8)	12873(8)	60(2)
C(15A)	2846(16)	−3913(8)	11869(7)	47(2)
C(16A)	4864(15)	−3433(7)	11099(7)	36(1)
N(1B)	5262(13)	392(6)	9421(6)	37(1)
C(1B)	7819(14)	948(7)	9056(6)	32(1)
C(2B)	8081(16)	2239(7)	9544(7)	39(2)
O(1B)	9424(13)	3224(6)	9056(6)	47(1)
N(2B)	6965(14)	2121(7)	10508(6)	38(1)
C(3B)	7190(17)	3138(8)	11166(7)	56(2)
C(4B)	5679(21)	2492(10)	12195(9)	101(3)
C(5B)	4038(17)	1354(9)	12026(7)	68(2)
C(6B)	5543(15)	888(8)	11085(6)	43(2)
C(7B)	4113(16)	292(7)	10415(7)	40(2)
O(2B)	1939(14)	−217(6)	10761(6)	57(1)
C(8B)	8569(15)	1154(7)	7912(6)	40(2)
C(9B)	7003(14)	2145(8)	7313(6)	35(1)
C(10B)	4781(15)	2726(7)	7649(6)	36(1)
N(3B)	3936(15)	3511(7)	6851(6)	47(2)
C(11B)	5708(17)	3456(8)	5969(7)	43(2)
C(12B)	5679(17)	4081(8)	4981(7)	56(2)
C(13B)	7610(20)	3863(9)	4232(8)	68(2)
C(14B)	9671(18)	3002(9)	4463(7)	66(2)
C(15B)	9618(17)	2394(8)	5464(7)	52(2)
C(16B)	7671(15)	2596(7)	6229(6)	37(2)

[a] $U_{eq} = 1/3\ \Sigma_i\ \Sigma_j\ U_{ij} a_i^* a_j^* (\mathbf{a}_i \cdot \mathbf{a}_j)$.

and m/z 283, respectively, i.e., the expected cyclic dipeptide form. The characteristic tryptophan side chain cleavage yielding the fragmentation m/z 130 is one of the highest observed fragment ions in the mass spectra. The ion at m/z 154 corresponds to the DKP-pyrrolidine fragment.

IR spectroscopy is a spectral method permitting reliable discrimination between *cis*- and *trans*-secondary amide bonds.[35–37]

The *cis*-amide bond nature of cyclo(Trp–Trp) is revealed through the specific values of ν(N—H) {3214.7 cm^{-1}} and ν(C=O) {1661.8 cm^{-1}}. The *cis*-amide bond also shows a further (amide III) absorption at 1333.2 cm^{-1} not shown by *trans*-amide bonds.[37]

For cyclo(Trp—Pro), the *cis* CONH group exhibits the amide I band at 1676 cm^{-1}. The amide II band (NH-in plane vibration) occurs at 1423.4 cm^{-1}. In addition, the *cis*-amide band shows a further (amide III) absorption at 1300.6–1313.7 cm^{-1}. The NH bending and CN stretching vibrations are observed at 1457.6 cm^{-1} and 1342 cm^{-1}, respectively. Although the presence of the amide band II (NH in-plane vibration) at 1550 cm^{-1} is characteristic of the *trans*-amide, its nonexistence in a spectrum does not imply the absence of all *trans*-amide bands.[37]

In cyclo(Trp–Trp) the Cβ resonance of L-tryptophan (29.88 ppm), is shifted upfield 0.82 ppm when compared to the same resonance in cyclo(L-Trp–L-Gly) (30.7 ppm) and cyclo(L-Leu–L-Trp)(30.7 ppm).[38] (Table 8). The four β and two α protons of cyclo(Trp–Trp) give rise to a single ABX pattern with the chemical shifts indicated in Fig. 4. The two kinds of β-protons differ by 0.51 ppm; the more shielded one has an apparent coupling to the α proton of 6.7 Hz, the less shielded, one of 4.2 Hz. If a folded conformation was important and persisted long enough to prevent averaging of this nonequivalence, there would be separated resonance patterns for the two kinds of methylene. Therefore, it seems that a preferred conformation is likely to be one in which each tryptophan residue shares the space over the DKP ring in such a fashion that the two β-methylenes have identical environments.

These findings are in agreement with the studies done on other aromatic cyclic dipeptides where it was revealed that diketopiperazines assume a flattened 2,5-piperazinedione ring conformation with the aromatic rings sharing the space over the piperazinedione nucleus, each aromatic residue being in a ''face to face'' position.[24] This type of conformation would support the energy transfer seen by Edelhoch *et al.*[9] A planar diketopiperazine, above which the indole rings of each tryptophan residue face each other, would allow π–π interactions between the indole rings.

Assuming an energetically preferred staggered conformation of the Cα—Cβ bond and approximate validity of Pachler's values[39] of J$\alpha\beta$ for J_{180} and J_{60} (13.6 Hz and 2.6 Hz, respectively) indicated that the preferred conformation for cyclo(Trp–Trp) is a folded one (49%) with the aromatic rings of trypto-

Table 5. Relevant Torsion Angles of Conformers 1 and 2 of cyclo(Trp–Pro)

Torsion angles[a]		Cyclo(Trp–Pro) 1	Cyclo(Trp–Pro) 2
C7A(7B)−N1A(1B)−C1A(1B)−C2A(2B)	φ_1	−43.739	−43.272
C2A(2B)−N2A(2B)−C6A(6B)−C7A(7B)	φ_2	−38.954	−38.056
N1A(1B)−C1A(1B)−C2A(2B)−N2A(2B)	Ψ_1	39.005	40.431
N2A(2B)−C6A(6B)−C7A(7B)−N1A(1B)	Ψ_2	34.682	35.415
C1A(1B)−C2A(2B)−N2A(2B)−C6A(6B)	ω_1	0.230	−1.017
C1A(1B)−N1A(1B)−C7A(7B)−C6A(6B)	ω_2	5.649	4.816
N1A(1B)−C1A(1B)−C8A(8B)−C9A(9B)	χ_1^{1A}	62.027	60.934
C1A(1B)−C8A(8B)−C9A(9B)−C16A(16B)	χ_1^{2A}	167.964	171.214
C8A(8B)−C9A(9B)−C10A(10B)−N3A(3B)		−177.379	−177.043
C16A(16B)−C9A(9B)−C10A(10B)−N3A(3B)		−0.294	0.983
C8A(8B)−C9A(9B)−C16A(16B)−C11A(11B)		178.497	177.865
C9A(9B)−C10A(10B)−N3A(3B)−C11A(11B)		−0.836	−1.270
N3A(3B)−C11A(11B)−C16A(16B)−C9A(9B)		−1.810	−0.449
C10A(10B)−C9A(9B)−C16A(16B)−C11A(11B)		1.274	−0.312
C10A(10B)−N3A(3B)−C11A(11B)−C16A(16B)		1.654	1.035
C12A(12B)−C11A(11B)−C16A(16B)−C15A(15B)		0.858	0.492
C14A(14B)−C15A(15B)−C16A(16B)−C11A(11B)		−1.839	−0.363
C16A(16B)−C11A(11B)−C12A(12B)−C13A(13B)		0.837	0.013
C13A(13B)−C14A(14B)−C15A(15B)−C16A(16B)		1.092	−0.221
C11A(11B)−C12A(12B)−C13A(13B)−C14A(14B)		−1.602	−0.624
C12A(12B)−C13A(13B)−C14A(14B)−C15A(15B)		0.680	0.744
C4A(4B)−C5A(5B)−C6A(6B)−N2A(2B)	χ_2^1	−34.820	−29.485
C3A(3B)−C4A(4B)−C5A(5B)−C6A(6B)	χ_2^2	35.372	29.047
N2A(2B)−C3A(3B)−C4A(4B)−C5A(5B)	χ_2^3	−21.142	−16.363
C6A(6B)−N2A(2B)−C3A(3B)−C4A(4B)	χ_2^4	−1.086	−3.009
C3A(3B)−N2A(2B)−C6A(6B)−C5A(5B)	θ	23.343	21.086
C2A(2B)−C1A(1B)−C8A(8B)−C9A(9B)		−61.969	−63.982
C1A(1B)−C8A(8B)−C9A(9B)−C10A(10B)		−15.424	−11.044

[a] Atom designation of conformer 1 with conformer 2 in brackets.

phan located above the DKP ring. The values for the two unfolded conformations were 37 and 15%, respectively. (Considering the α–β coupling values for cyclic peptides, equal couplings near 3 Hz may be expected for the folded conformation, whereas one large (14 Hz) and one small (3 Hz) coupling would present a single, unfolded conformation. If the two unfolded conformations are equally populated and the folded form is negligibly so, the two couplings

Table 6. Hydrogen Bonding Scheme for cyclo(Trp–Trp)

D−H---A[a]	D−A	H---A	Angle D−H---A
N4−HN4−O3	2.913 Å	2.345 Å	110.0°
N3−HN3−O3	2.928 Å	2.155 Å	137.3°
N1−HN1−O1	2.986 Å	1.935 Å	163.3°
N2−HN2−O2	2.994 Å	2.059 Å	143.2°

[a] D-donor, H-hydrogen, A-acceptor.

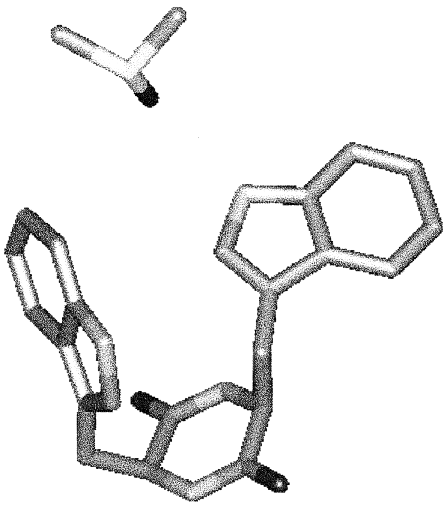


Fig. 3. Molecular modeling of cyclo(Trp–Trp)·C_2H_6SO.

Table 7. Hydrogen Bonding Scheme for cyclo(Trp–Pro)

D−H−−A[a]	D−A	H−−A	Angle D−H−−A
N3A−HN3A−O2B	2.903 Å	2.257 Å	116.4°
N3B−HN3B−O2A	2.902 Å	2.244 Å	117.2°
N1A−HN1A−O1B	3.085 Å	2.215 Å	136.1°
N1B−HN1B−O1A	3.094 Å	2.087 Å	147.0°

[a] D-donor, H-hydrogen, A-acceptor.

should be equal and about 8 Hz. If however, all three conformations are equally populated, the couplings should be equal and near 6.5 Hz.)[24]

For cyclo(Trp–Trp), no chemical shift change is detected in the indole ring as a consequence of the proximity of the two aromatic residues.

In DMSO-d_6 solution (Table 9), the two β-protons of the tryptophan residue of cyclo(Trp–Pro) differ by 0.18 ppm, the more shielded has an apparent coupling to the α proton of 5.7 Hz, the less shielded, one of 4.8 Hz. A predominance (52%) of the folded conformation for the tryptophan side chain in DMSO-d_6 was estimated using Pachler's[39] analysis. Judging from the ^3J(Trp-α,β) coupling constants, the tryptophan side chain prefers the unfolded conformation (73%) in $CDCL_3$. There is a shift of the signal Cα-H (Trp) (4.29 ppm) to lower field in comparison with the corresponding resonance in cyclo(Trp-Trp) (3.87 ppm). The Cγ proton resonance of proline in DMSO-d_6 (1.65 ppm) is shifted upfield when compared with the same resonances in cyclo(Phe–Pro) (1.72 ppm) and cyclo(Tyr–Pro) (1.70 ppm).

Table 8. The ^{13}C NMR Data of cyclo(Trp–Trp)[a]

Carbon atom	ppm
Trp-β	29.88
Trp-α	55.24
Trp–Ar (C_3)	108.85
Trp–Ar (C_7)	111.29
Trp–Ar (C_4)	118.41
Trp–Ar (C_5)	118.60
Trp–Ar (C_6)	120.85
Trp–Ar (C_2)	124.47
Trp–Ar (C_8)	127.45
Trp–Ar (C_9)	136.17
C=O	166.89

[a] The assignments of the indole ring of tryptophan are based on those of Parker and Roberts,[42] and Deslauriers *et al.*[38]

For cyclo(Trp–Pro), a low field ^{13}C shift of the Cβ resonance of L-tryptophan (25.74 ppm, DMSO-d_6, 26.76 ppm, $CDCL_3$) (Table 10) was observed in comparison with the corresponding resonances seen in the spectra of cyclo(Trp–Trp) (29.88 ppm, DMSO-d_6) and cyclo(Trp–Leu) (30.7 ppm, DMSO-d_6)[38]. The Cα resonance of tryptophan (55.20 ppm, DMSO-d_6) is shifted upfield when compared with the same resonance in cyclo(L-Leu–L-Trp)(57.1 ppm, DMSO-d_6) and cyclo(L-Trp–Gly) (57.0 ppm, DMSO-d_6)[38]. From the ^{13}C NMR data shown in Table 10 it is seen that for cyclo(Trp–Pro), the value of $\Delta\delta(\beta\gamma)$ is nearly constant regardless of solvent (5.8 in DMSO-d_6 and 5.7 in $CDCl_3$). Assuming, however, that the $\Delta\delta(\beta\gamma)$ value reflects an equilibrium state between the planar and boat forms of this compound, expressed by the equilibrium constant K = [planar form]/[boat form] and remembering that for the planar form ($\theta = 60°$) $\Delta\delta(\beta\gamma) = 7.33$ ppm and for the boat form ($\theta = 30°$) $\Delta\delta(\beta\gamma) = 4.90$ ppm, the amounts of both conformers can be calculated by the equation: 5.8 or 5.7 = 4.90a + 7.33(1-a) where a and (1-a) are the molar fractions of boat and planar forms, respectively. Using this procedure, the values of 0.63 (boat) and 0.37 (planar form) in DMSO-d_6 and 0.67 (boat) and 0.33 (planar form) in $CDCl_3$ were obtained.[40] These results support the stability of the boat conformation for this proline-containing cyclic dipeptide.

In a slightly different approach to the method of preparation described in the experimental section, the N-protected dipeptide ester (Boc–Pro–Trp–OMe) was treated with formic acid (20 ml) containing 0.2 ml anisole, to remove the boc-group. The resulting unprotected dipeptide formate salt was dissolved in a neutral medium [toluene-*sec*-butanol (1:4)] and refluxed in an oil bath (120°C) for 3 h.[41] After concentrating the solution to 8 ml and cooling to 0°C the products were filtered off and recrystallized from chloroform-n-hexane.

Two conformations of cyclo(Pro–Trp) crystallized in the space group $P1$ with cell dimensions a = 5.404(1), b = 9.888(2), c = 13.438(2) Å, α = 80.45(1), β = 78.67(1), and γ = 89.09(2)°. The conformation of the backbone, the orientation of the aromatic side chain, the puckering modes for the pyrrolidine rings, and the hydrogen bonding of these conformers are to a certain extent similar, although not identically to that of cyclo(Trp–Pro) [(conformer 1: φ_1 = −46.229, φ_2 = −36.307, Ψ_1 = 40.683, Ψ_2 = 33.171, ω_1 = −1.367, ω_2 = 7.574, χ_1^{1A} = 60.327, χ_1^{2A} = 167.584,

Proton	ppm
Trp-β (J_g = 14.3 Hz, J_v = 6.7 Hz)	2.20
Trp-β (J_g = 14.3 Hz, J_v = 4.2 Hz)	2.71
Trp-α	3.87
Trp-Ar	6.61
Trp-Ar	6.94
Trp-Ar	7.04
Trp-Ar	7.28
Trp-Ar	7.35
NH	7.65
NH (Indole)	10.81

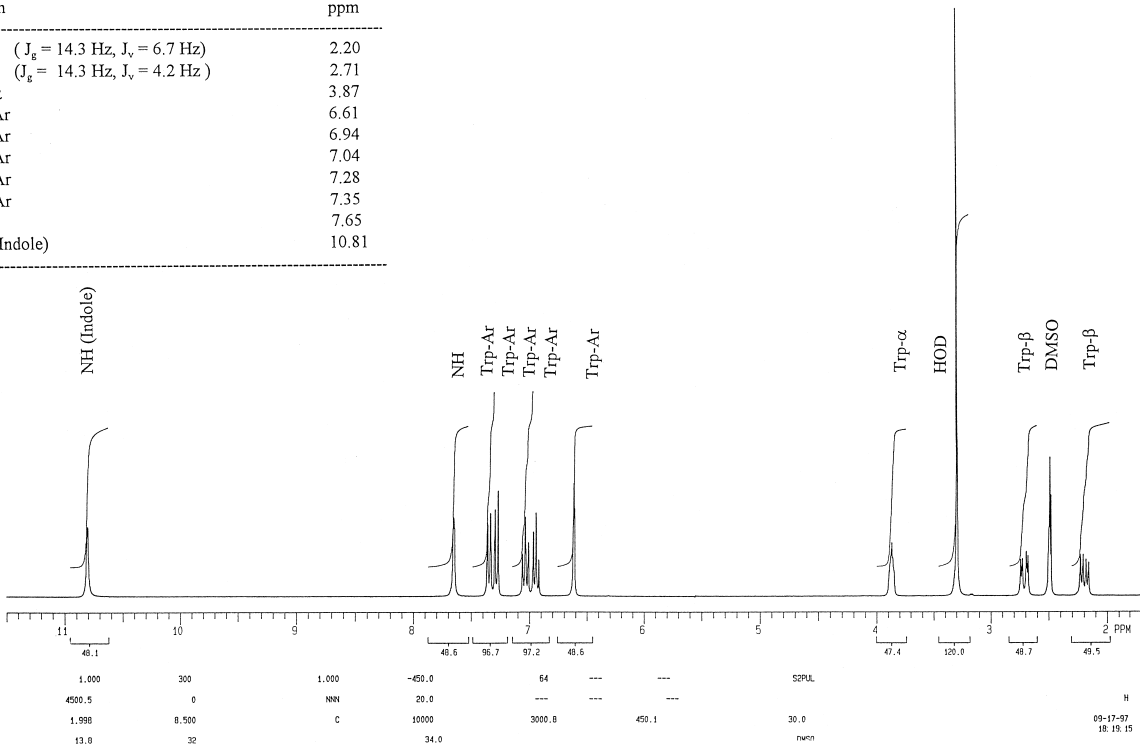


Fig. 4. [1]H NMR spectrum of cyclo(Trp–Trp).

Table 9. [1]H NMR Data of cyclo(Trp–Pro)[a]

Proton	$CDCL_3$ ppm	DMSO-d_6 ppm
NH (Indole)	8.35	10.85
NH	5.72	7.67
Trp–Ar	7.57	7.56
Trp–Ar	7.37	7.32
Trp–Ar	7.21	7.18
Trp–Ar	7.12	7.05
Trp–Ar	7.06	6.96
Trp-α	4.35	4.29
Pro-α	4.04	4.05
Trp-β	3.73	3.24
Pro-δ	3.59 (2H)	3.37
Pro-δ	—	3.29
Trp-β	2.96	3.06
Pro-β	2.29	1.97
Pro-γ	1.99	1.65 (2H)
Pro-β	1.94	1.39
Pro-γ	1.87	—

[a] ^2J(Trp-β) = 15 Hz, ^3J(Trp-α,β) = 5.7 Hz and 4.8 Hz)(DMSO-d_6). ^2J(Trp-β) = 15 Hz, ^3J(Trp-α,β) = 10.7 Hz and 3.8 Hz)($CDCL_3$).

Table 10. [13]C NMR Data of cyclo(Trp–Pro)[a]

Carbon atom	$CDCL_3$ ppm	DMSO-d_6 ppm
Pro-γ	22.48	21.75
Trp-β	26.76	25.74
Pro-β	28.19	27.55
Pro-δ	45.33	44.52
Trp-α	54.60	55.20
Pro-α	59.18	58.38
Trp–Ar (C_3)	109.99	109.36
Trp–Ar (C_7)	111.61	111.27
Trp–Ar (C_4)	118.55	118.28
Trp–Ar (C_5)	120.04	118.69
Trp–Ar (C_6)	122.81	120.93
Trp–Ar (C_2)	123.39	124.45
Trp–Ar (C_8)	126.85	127.44
Trp–Ar (C_9)	136.81	136.10
Pro–C=O	165.69	165.65
Trp–C=O	169.48	169.13

[a] The assignments of the indole ring of tryptophan are based on those of Parker and Roberts, 1970,[42] but resonances C_5 and C_6 have been interchanged based on studies of indoles deuterated in position 5 and position 6.[38]

Table 11. Hydrogen Bonding Scheme for cyclo(Pro–Trp)

D−H−−A[a]	D−A	H−−A	Angle D−H−−A
N2A−HN2A−O2B	3.078 Å	2.138 Å	130.7°
N3A−HN3A−O1B	2.877 Å	2.182 Å	119.8°
N2B−HN2B−O2A	3.023 Å	2.092 Å	121.5°
N3B−HN3B−O1A	2.914 Å	2.260 Å	120.4°

[a] D-donor, H-hydrogen, A-acceptor.

$\chi_2^1 = -34.274$, $\chi_2^2 = 37.275$, $\chi_2^3 = -24.064$, $\chi_2^4 = 1.508$, $\theta = 20.483$); (conformer 2: $\varphi_1 = -44.902$, $\varphi_2 = -42.014$, $\Psi_1 = 40.900$, $\Psi_2 = 38.133$, $\omega_1 = 1.729$, $\omega_2 = 4.587$, $\chi_1^{1A} = 59.562$, $\chi_1^{2A} = 172.769$, $\chi_2^1 = -36.683$, $\chi_2^2 = 34.086$, $\chi_2^3 = -17.941$, $\chi_2^4 = -4.944$, $\theta = 26.476$)].

The DKP ring of cyclo(Pro–Trp) for both conformers can be considered a typical boat conformation. The tryptophan side chains of these conformers are folded toward the diketopiperazine (DKP) ring. The pyrrolidine ring for conformer 1 can be described as an envelope (Cs−Cβ-*endo*) conformation[34] in contrast to the pyrrolidine ring symmetry for conformer 2 which is an intermediate[34] between C_s and C_2 with Cβ-*endo* and Cγ-*exo* with respect to C′. The two prolyl rings are puckered at the β-carbon atoms which deviate from the best planes defined by the four remaining atoms. The crystal structures are stabilized by four intermolecular hydrogens bonds (Table 11). Very evident is the difference in the angles of donor-hydrogen-acceptor and the lengths of the hydrogen bonds between cyclo(Trp–Pro) and cyclo(Pro–Trp).

Molecular modeling (Fig. 5) showed that there are great similarities between conformer 1 of cyclo(Trp–Pro) and conformer 1 of cyclo(Pro–Trp). Similarities were also observed between conformer 2 of cyclo (Trp–Pro) and conformer 2 of cyclo(Pro–Trp). The difference is mainly between conformers 1 and conformers 2 due to the puckering of the pyrrolidine ring (C_s compared to an intermediate between C_s and C_2).

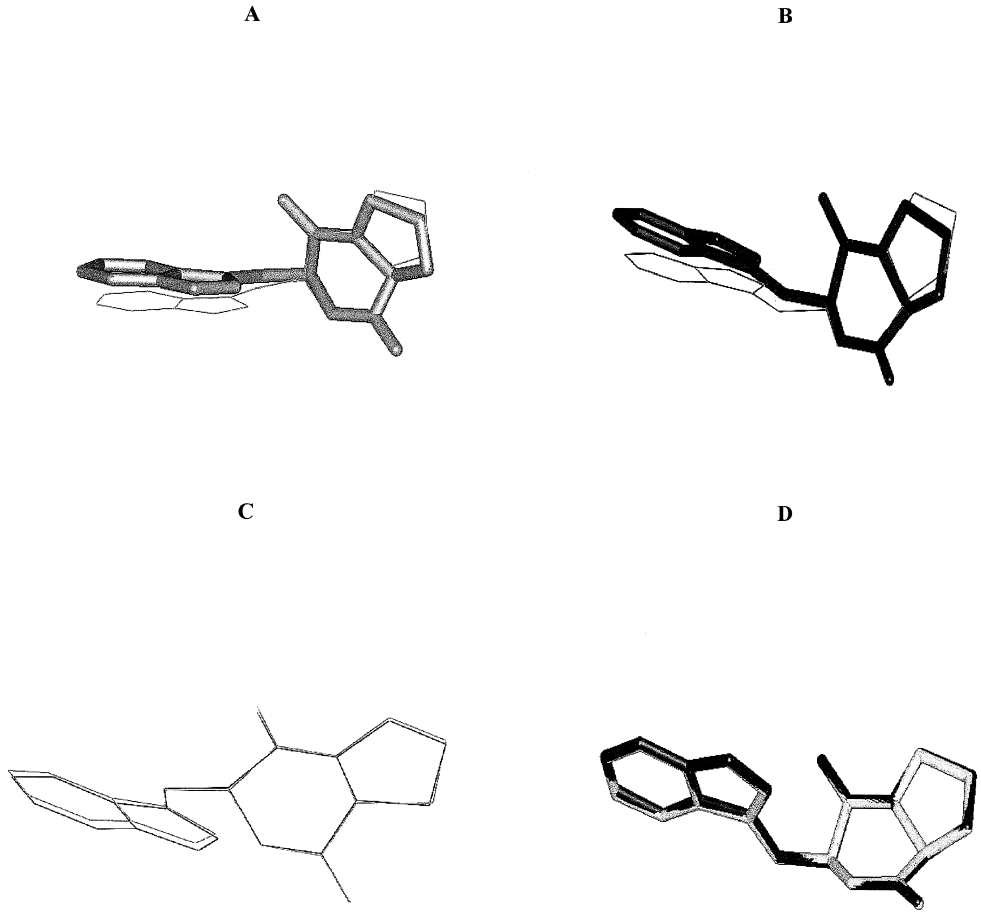


Fig. 5. Molecular modeling of cyclo(Trp–Pro) and cyclo(Pro–Trp).

Data Given in Figure 5 (A, B, C, D)

A. Molecular fit of cyclo(Trp–Pro)[conformer 1: (gray line rendering), conformer 2: (gray stick rendering)]

B. Molecular fit of cyclo(Pro–Trp)[conformer 1: (black line rendering), conformer 2: (black stick rendering)]

C. Molecular fit of cyclo(Trp–Pro)[conformer 1: (gray line rendering)] and cyclo(Pro–Trp)[conformer 1: (black line rendering)]

D. Molecular fit of cyclo(Trp–Pro)[conformer 2: (gray stick rendering)] and cyclo(Pro–Trp) [conformer 2: (black stick rendering)]

Biological studies on cyclo(Trp–Trp), cyclo (Trp–Pro), and two other selected aromatic diketopiperazines indicated that these cyclic dipeptides exhibit biological activity in both prokaryotes and eukaryotes. Three of the cyclic dipeptides block cation channels in ventricular myocytes, whilst all increase the expression of alkaline phosphatase. All of the cyclic dipeptides exhibit concentration dependent antibacterial properties. (Another 25 cyclic dipeptides are currently being tested and evaluated for biological activity.)

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