A hybrid cyclic bisproline designed to adopt a β-fold: crystal structure of cyclo(ProNHCH$_2$CH$_2$NHProCOCH$_2$CH$_2$CO)

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The crystal structure of 14-membered cyclo(ProNHCH$_2$CH$_2$NHProCOCH$_2$CH$_2$CO) reveals the presence of an internal NH···O=C bond dividing the molecule into two halves of 10-membered hydrogen-bonded rings; the molecules self-assemble into cyclic dimers through a symmetrical pair of intermolecular NH···O=C hydrogen bonds; a layered structure is formed, alternating layers of cyclic dimers and layers of chloroform molecules, all of which make strong CH···O hydrogen bonds with the cyclic dimers.

Design of conformationally constrained peptides mimicking receptor-bound conformation is an area of intense current interest in drug design.[1] It is generally believed that a β-folded structure is the most likely conformation present at the active site of many naturally occurring peptides and proteins.[2] Among the natural amino acids, proline is reported to be the most prevalent at the turn locations.[3] In recent years several designs of multiple-stranded β-sheets have been reported[4] using a Pro-Gly motif as the turn inducer. An attractive approach to β-turn folds would be to cyclize proline-containing peptides with a rigid moiety that may force the peptide chain to fold and run in an antiparallel direction. We demonstrate herein the first illustration of this concept and report on the crystal structure of a hybrid cyclic peptide with repeats of L-proline and dimethylene units in a 14-membered ring. The target cyclic peptide **1** was prepared by a two-step procedure (Scheme 1) involving first the formation of a core-modified Z–proline bispeptide which on deprotection and condensation with succinyl chloride under a high-dilution condition yielded the desired cyclic peptide in good yields.[5]

Suitable crystals for **1** were obtained from chloroform solution by slow diffusion of hexane vapour. The colourless shining crystals were found to crumble into an opaque powdery solid when exposed to air. For this reason diffraction measurements were carried out at low temperature on a crystal covered with immersion oil.

The crystal structure[6] of **1** showed the presence of two independent molecules A and B with very similar conformations. The contents of the unit cell also included three chloroform molecules. Molecule A [Fig. 1(a)] and B [Fig. 1(b)] each contain an intramolecular NH···O=C hydrogen bond dividing the macrocycle into two equal halves each enclosing a 10-membered hydrogen-bonded ring. The molecules A and B are further engaged in a dimer formation [Fig. 2(a)] through a similar pair of intermolecular NH···O=C hydrogen bonds. As shown in [Fig. 2(b)] each cyclic dimer is surrounded by three $CHCl_3$ molecules that make strong C–H···O hydrogen bonds with carbonyls O18, O18s and O3s of both macrocycles, and by additional $CHCl_3$ molecules from neighboring cells. Molecule A participates in C–H···O hydrogen bonding only with one of the $CHCl_3$ molecules while molecule B makes C–H···O contacts with two $CHCl_3$ molecules. Table 1 presents the hydrogen bond parameters. The dimers further assemble in infinite columns extending into a layered structure wherein sheets of $CHCl_3$ molecules (three for each dimer) alternate with columns of dimers [Fig. 2(b)]. The chloroform molecules among themselves make only van der Waals contacts. The

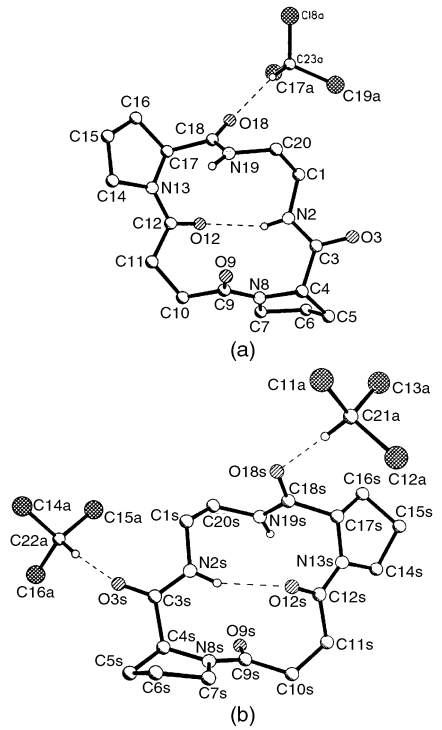
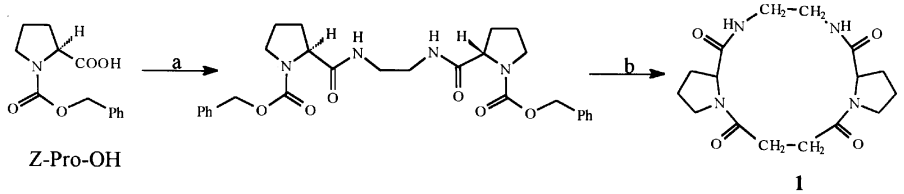


Fig. 1 Molecule A (a) as well as molecule B (b) are each engaged in intramolecular NH···O=C hydrogen bonding dividing the macrocycle into two equal halves of 10-membered hydrogen-bonded rings.



Scheme 1 *Reagents and conditions*: $NH_2CH_2CH_2NH_2$, diphenyl phosphoryl azide (DPPA), DMF–CH_2Cl_2; b, (i) Pd/C 5%, H_2, (ii) $ClCOCH_2CH_2COCl$, NEt_3, CH_2Cl_2.

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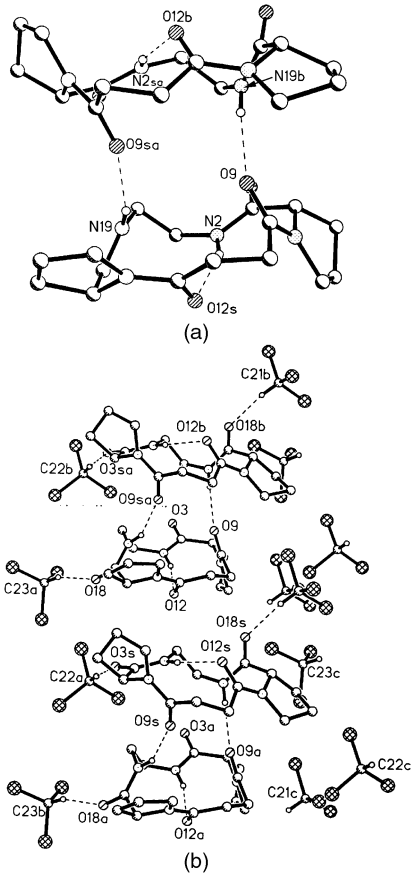

Fig. 2 (a) Hydrogen-bonded cyclic dimer of molecule A and B formed through a similar pair of NH···O=C hydrogen bonds. (b) Packing of dimer columns into a layered structure. Each dimer is surrounded by three $CHCl_3$ molecules that make strong C–H···O contacts with the macrocycles. The remaining $CHCl_3$ molecules in the layers are from neighboring unit cells.

Table 1 Hydrogen bonds

Type	Donor	Acceptor	D–A/Å	H[a]–A/Å	DH···O/°
4 → 1	N2	O12	3.104	2.25	159
4 → 1	N2s	O12s	3.041	2.19	158
Dimer	N19	O9s[b]	2.959	2.12	154
Dimer	N19s	O9[c]	3.071	2.18	172
CH–O	C23	O18	3.085	2.16	160
CH–O	C21	O18s	3.081	2.13	170
CH–O	C22	O3s	3.001	2.04	175

[a] Hydrogen atoms were placed in idealized positions at N–H = 0.90 Å and C–H = 0.96 Å. [b] At symmetry equivalent: *x*, −1 + *y*, *z*. [c] At symmetry equivalent: *x*, 1 + *y*, *z*.

presence of such a large proportion of chloroform molecules in the crystal structure of **1** is rather unusual and may be attributed to the hydrophobic nature of the proline macrocycle.

The β-turns in the proline macrocycle (Fig. 1), mimic true βII′- and βIII-turns in standard peptides, despite a substitution of C1 (methylene) for a N atom in the upper half of the macrocycle and a substituton of C11 for a N atom in the lower half. An inspection of the torsional angles listed in Table 2 shows that the upper half closely resembles a βII′-type turn, whereas the lower half has angles resembling a βIII-type turn.

^{1}H NMR variable-temperature (VT) studies showed a value of −3.75 ppb K^{-1} for the temperature coefficient indicating a moderate amount of intramolecular hydrogen bonding in DMSO solvent. ROESY studies in DMSO-d_6 did not show any significant cross-peaks except weak interaction between NH and methylene spacer units. The presence of dimeric structures was also indicated by electrospray mass spectroscopy.

In conclusion, incorporation of CH_2CH_2 units in an alternating sequence with Pro units in a ring seems to lead to preference

Table 2 Conformation angles (°)

Angle	Molecule A	Molecule B	Std. label[a]	Idealized turns[b] βII′	Idealized turns[b] βIII
C10C11C12C13	167	170	ω_o	180	
C9C10C11C12	61	63	ϕ_1	60	
N8C9C10C11	−138	−138	ψ_1	−120	
C4N8C9C10	−179	−179	ω_1	180	
C3C4N8C9	−79	−80	ϕ_2	−80	
N2C3C4N8	1	3	ψ_2	0	
C1N2C3C4	174	179	ω_2	180	
C11C12N13C17	−178	179	ω'_2		
C12N13C17C18	−60	−49	ϕ_3		−60
N13C17C18N19	−26	−40	ψ_3		−30
C17C18N19C20	171	176	ω_3		180
C18N19C20C1	−66	−62	ϕ_4		−60
N19C20C1C2	−60	−58	ψ_4		−30
C20C1N2C3	−103	−109	X_4		

[a] Conventions for normal peptides in ref. 7. Labeling of torsional angles with the standard ϕ, ψ and ω symbols, and μ or θ for angles about the CH_2–CH_2 is complicated since the order of the backbone atoms in C20 to C11 is in the retro direction as compared to C1 to C10. In the pseudo 4 → 1 turns, atoms C10, C4 and C17, C20 are in the corner positions (C^α atoms) of two standard β-turns. The ϕ, ψ and ω labels were chosen so that torsional angles in the pseudo 4 → 1 turns could be compared directly to standard types of 4 → 1 β-turns. [b] Idealized values in ref. 8.

of a C_{10} hydrogen-bonded turn structure. The presence of all-*trans* amide bonds in the constrained 14-membered ring of **1** and an unusually large amount of chloroform molecules stabilizing the structure through C–H···O hydrogen bonds are additional noteworthy features. The design of related hybrid peptides containing an increasing number of proline units in the ring is in progress.

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5 *Selected data* for **1**: yield 60%; mp 264–269 °C; δ_H(500 MHz, DMSO-d_6) 1.76–1.92 (m, 6H), 2.06–2.16 (m, 2H), 2.25–2.36 (m, 2H), 2.76–2.88 (m, 4H), 3.36 (t, 2H), 3.54 (m, 2H), 3.77 (m, 2H), 4.18 (q, 2H), 6.86 (d, *J* 7.5 Hz, 2H), ES-MS *m/z* (%) 337(5) $(MH)^+$, 359 (100) $(M + Na^+)$, 375(92) $(M + K^+)$, 695(92) $(2M + Na^+)$, 711(20) $(2M + K^+)$.

6 *Crystal data* for **1**: $2[C_{16}H_{24}N_4O_4]\cdot 3CHCl_3$, space group *P*1, *a* = 10.141(1), *b* = 10.892(1), *c* = 12.718(1) Å, α = 67.50(1), *β* = 81.87(1), *γ* = 63.92(1)°, *V* = 1165.1(2) Å^3, D_c = 1.469 g cm^{-3}, Cu-Kα radiation, *λ* = 1.54178 Å, Least-square refinement on F^2, R_1 = 0.083, wR_2 = 0.202. Data collection at −60 °C; crystal covered with microscope oil (severe solvent loss at 20 °C with crystal removed from mother liquor). CCDC 182/1885.

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