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Synthesis and Structure of Symmetrical Bicyclic Hexapeptides Bridged by Metathesis Reaction

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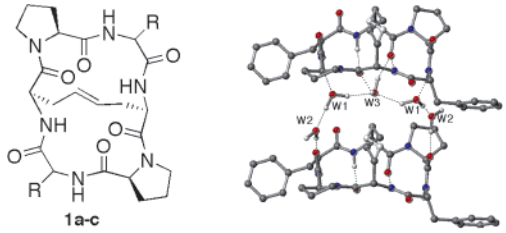
ABSTRACT



Bicyclic hexapeptides 1a–c were synthesized via an intramolecular ring-closing metathesis reaction on solid phase followed by an N- to C-terminal cyclization in solution. Structural elucidation showed that these compounds assumed a C_2-symmetrical structure with two β-turns. The *trans*-ethylene plane was found to occupy two positions in rapid interconversion. One of the bicyclic hexapeptides crystallized with five water molecules, which made an arch above the ethylene group.

The synthesis of rigid molecules and particularly in peptide chemistry has generated a great deal of interest over the past few years because the reduction of conformational freedom often results in higher receptor binding affinity and/or modulation in biological activity. Although cyclic peptides represent good candidates for conformational restriction,[1] they still exhibit significant flexibility. Extra conformational restriction is necessary to introduce stronger conformational constraint. Usually, this is achieved by a second cyclization through disulfide or lactam bridge formation. As an example, a cyclic hexapeptide-based tachykinin NK2 receptor antagonist, after a second cyclization via a lactam, resulted in a 10−100 fold increase in activity.[2,3] It is well established that the so-called "bivalent ligand" approach can lead to agonists or antagonists of peptide receptors with a considerably increased potency/selectivity ratio.[4] This concept has been extensively applied to several peptide hormones.[5] Moreover, several receptors, including tyrosine kinase and the cytokine receptor superfamily, require dimerization for signal transduction.[6] These reports emphasize the significance of designing new dimeric compounds. β-Turns play an important role in many biological molecular recognition events.[7,8] Among structures that should mimic β-turns, cyclic hexapeptides constitute interesting lead compounds.[9,10] In this study, we were stimulated to design bivalent, small, rigid β-turn mimics

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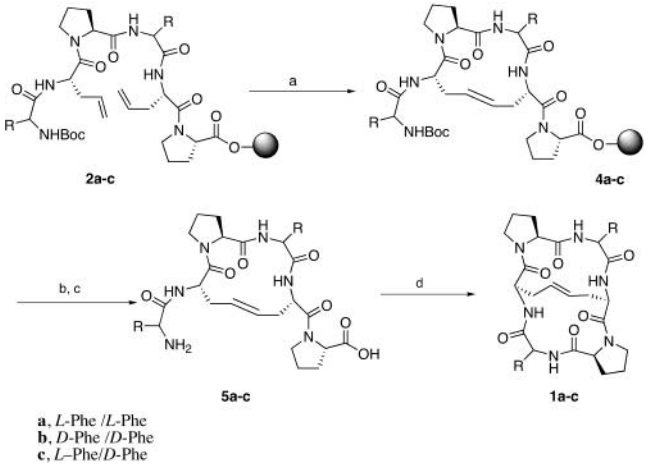
based on bicyclic hexapeptides having C_2-symmetry. We focused our attention on cyclic hexapeptides bridged by functions that disrupt neither the peptide backbone conformation by supplementary interaction nor the molecular C_2-symmetry.

The disulfide bond between Cys residues and the lactam group between Asp and Dap residues are usually used to create conformational constraints, but the asymmetry of the former and the ability of the latter to interact with the peptide groups may induce asymmetrical global conformations. The chiral disulfide group in cyclo(Cys-Gly-Pro-Phe)$_2$ results in two Cys residues having different side chain rotamers and two Pro-Phe sequences assuming the type I and type II β-turns in the crystal.[11] On the other hand, the lactam bridge in the pseudosymmetrical cyclohexapeptide cyclo(Phe-Asp-Trp-Phe-Dap-Trp)cyclo(2β-5β) interacts with the Trp-Phe amide bond so that the type I and type II β-turn structures are adopted by the Trp-Phe sequences in the solid state.[12] These conformations are retained in solution.

Contrary to the lactam or disulfide-bridge, the trans carbon−carbon double bond presents the advantage of keeping symmetry in the molecule and possesses a low probability of interaction with the molecular backbone. We then focused our effort on ring-closing metathesis (RCM), which has found extensive use for the synthesis of cyclic compounds.[13−17] However, as far as we know there is no example in the literature of cyclic compounds with the additional constraint of a carbon−carbon double-bond bridge. We focused on the synthesis of constrained compounds **1a**−**c**, which differ in the L- or D-stereochemistry of the Phe residue (Scheme 1).

Scheme 1. Synthesis of Bicyclohexapeptides **1a**−**c**



[a] $Cl_2(PCy_3)Ru{=}CHPh$ (5 mol %), CH_2Cl_2, 40 °C, 24 h. [b]TFA/CH_2Cl_2. [c]HF/anisole, 0 °C. [d]HBTU, HOBt, DIEA, DMF.

These molecules were obtained by an intramolecular RCM reaction on solid phase followed by an N- to C-terminal cyclization in solution to produce molecules possessing C_2-symmetry (**1a** and **1b**). The conformational preferences of **1a**−**c** in solution were studied by proton NMR, and the crystal structure of **1b** was solved by X-ray diffraction.

The synthesis of the alkene-containing linear hexapeptides **2a**−**c** was accomplished by conventional solid-phase peptide synthesis (Scheme 1) using Boc chemistry on a Boc-Pro-Merrifield resin (substitution 0.6 mmol/g).

Boc-L-(or D)-Phe-OH and Boc-Pro-OH were coupled with benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) reagent and *N*-Boc-allylglycine (Boc-allylGly-OH) with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). Resin−linear hexapeptides were treated with 5 mol % Grubbs's Ru-catalyst **3** in CH_2Cl_2 for 48 h at 40 °C to yield compounds **4a**−**c**. After deprotection of the Boc group by TFA and cleavage from the resin using standard liquid HF procedure (10 mL of 9:1 HF/anisole per gram of peptidyl resin, 1 h at 0 °C), the cyclic compounds **5a**−**c** were obtained. Their analyses by HPLC and mass spectrometry coupled to liquid chromatography (LC/MS) revealed total conversion of the acyclic compounds.[18] The second cyclization step was performed in dilute DMF (1 mM) with DIEA, using HBTU as a coupling reagent and *N*-hydroxybenzotriazole (HOBt) as an additive, for 4 h. Under these conditions, compounds **5a**−**c** afforded bicyclic compounds **1a**−**c** in 80% conversion and 50% yield after HPLC purification.

Compound **1b** crystallized with five water molecules (Figure 1).[19] The peptide backbone assumed a rigid C_2-symmetrical structure, a classical structure for symmetrical hexapeptides, and contained two Pro-D-Phe sequences folded

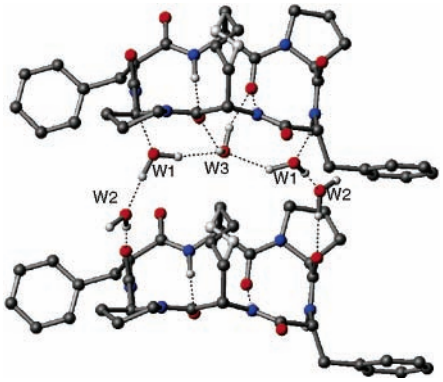


Figure 1. X-ray diffraction structure of cyclo(D-Phe-L-Allgly-L-Pro-D-Phe-L-Allgly-L-Pro)cyclo(2−5) **1b**. The conformation A and B of the ethylene bridge are represented in dark and light gray, respectively. The hydrogen bonds are shown as dashed lines.

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Table 1. Main Torsional Angles for A and B Forms of **1b** in the Crystal

			allylGly*	
torsional angles[a]	Pro	D-Phe	A	B
ϕ	−65	78	−149	
ψ	117	9	172	
ω	180	−172	175	
χ^1	−25	68	83	46
χ^2	37	−97/82	−94	92
χ^3	−34		170	−171
χ^4	19			

[a] According to the usual IUPAC-IUB nomenclature.[20]dHBTU, HOBt, DIEA, DMF

Table 2. Nature and Length (Å) of the H−Bonds for **1b**

donor	acceptor	distance
allylGly*-NH	allylGly*-CO	3.12
D-Phe-NH	W1	2.87
W1H	W3	2.81
W1H′	W2	2.68
W2H	D-Phe-CO	2.76
W2H′	Pro-CO	2.74
W3H	allylGly*-CO	2.78

in a type II β-turn (Table 1). The ethylene carbons accommodated two dispositions, A and B, in a 65:35 ratio (Figure 1). There is no direct peptide−peptide interaction, and each molecule was surrounded by five water molecules (Table 2) making an archway above the ethylene group.

In acetone-d_6, **1a** and **1b** gave only one set of proton resonances, indicating that both molecules retain C_2-symmetrical conformations (Table 3). On the other hand, the ^{1}H NMR spectrum for the nonsymmetrical compound **1c** was practically the addition of that of **1a** and **1b**, so that each half of molecule **1c** retained the same conformations as in the symmetrical molecules **1a** and **1b**. In all three cases, the allylGly*-N*H* resonances were much less affected than the Phe-N*H* resonances by addition of DMSO-d_6, indicating that the Pro-Phe and Pro-D-Phe sequences were β-folded.

The high $J_{N\alpha}$ coupling constant (8.4−8.9 Hz) for both L- and D-Phe residues indicated a βI-folded Pro-Phe sequence in both **1a** and **1c** and a βII-folded Pro-D-Phe sequence in both **1b** and **1c**.[21]

Due to molecular symmetry, the (*Z*)- or (*E*)-conformation of the ethylene bond could not be determined in solution for both **1a** and **1b**. However, a vicinal coupling constant of 15.7 Hz, typical for an (*E*)-ethylene bond,[22] was measured for **1c** using irradiation of the allylGly*-C$^\beta$*H*$_2$ resonances. On the other hand, irradiation of the ethylene C*H* resonances gave access to the allylGly* $J_{\alpha\beta}$ couplings. Their small values (3.4−3.9 Hz) would account for the gauche side chain rotamer corresponding to $\chi^1 = 60°$,[23] but such a disposition, implying that the ethylene plane was perpendicular to the peptide ring, must be rejected because of severe hindrances. We therefore concluded that the $J_{\alpha\beta}$ coupling constants were in fact average values corresponding to the two rapidly interconverting orientations A and B of the ethylene group, already observed in the crystal of **1b**. On the basis of the two torsional angles χ^1 in Table 1 and of the $J_{\alpha\beta}$ vs χ^1 correlation,[23] the A:B ratio is estimated to be about 60:40, quite close to that in the crystal structure of **1b**.

Table 3. Chemical Shifts (ppm) and Coupling Constants (Hz, in Parentheses) for Compounds **1a**−**c** in Me_2CO-d_6

compound		**1a**	**1b**	**1c**
Pro	C$^\alpha$H	4.35		4.22
	C$^\beta$H$_2$ + C$^\gamma$H$_2$	1.8−2.3		1.9−2.3
	C$^\delta$H$_2$	3.95/3.72		3.85/3.60
L-Phe	NH	7.94 (8.4)		7.77 (8.4)
	C$^\alpha$H$_X$	4.08		3.97
	C$^\beta$H$_A$H$_B$ ($J_{AX}/J_{BX}/J_{AB}$)	3.38/3.36 (6.8/8.0/−13.1)		3.39/3.34 (4.2/11.3/−13.7)
	Ph	7.3−7.5		7.3−7.5
allylGly	NH	7.94 (7.7)		7.85 (7.9)
	C$^\alpha$H$_X$	4.89		4.79
	C$^\beta$H$_A$H$_B$ ($J_{AX}/J_{BX}/J_{AB}$)	2.47/2.32 (3.4/3.6/−13.1)		2.42/2.28 (3.7/3.6/−13.1)
	C$^\gamma$H$_Y$ = ($J_{AY}/J_{BY}/J_{YY'}$)	5.58 (4.9/5.1)		5.37 (5.4/7.3/15.7)
Pro	C$^\alpha$H		4.43	4.38
	C$^\beta$H$_2$ + C$^\gamma$H$_2$		1.9−2.4	1.8−2.3
	C$^\delta$H$_2$		3.81/3.51	3.80/3.50
D-Phe	NH		7.80 (8.6)	7.80 (8.9)
	C$^\alpha$H$_X$		4.46	4.42
	C$^\beta$H$_A$H$_B$ ($J_{AX}/J_{BX}/J_{AB}$)		3.21/2.99 (3.3/11.0/−14.3)	3.20/2.98 (3.3/11.3/−14.2)
	Ph		7.3−7.5	7.3−7.5
allylGly	NH		7.95 (8.1)	7.94 (8.1)
	C$^\alpha$H$_X$		4.81	4.78
	C$^\beta$H$_A$H$_B$ ($J_{AX}/J_{BX}/J_{AB}$)		2.50/2.23 (3.8/3.5/−13.2)	2.50/2.22 (3.9/3.5/−13.0)
	C$^\gamma$H$_Y$ = ($J_{AY}/J_{BY}/J_{YY'}$)		5.21 (5.0/7.9)	5.29 (5.6/7.4/15.7)

In conclusion, both symmetrical compounds **1a** and **1b** contain two β-turns of type I and II, respectively, thus confirming our initial postulate that such a bicyclic structure could be used for the design of C_2-symmetrical molecules. The trans planar structure of the ethylene bridge is demonstrated in the crystal and in solution by NMR analysis of the nonsymmetrical derivative **1c**, but it adopts two orientations with reference to the peptide backbone.

The intrinsic C_2-symmetry of the trans ethylene group and the absence of strong interactions with the main chain are probably responsible for the global C_2-symmetry of molecules **1a** and **1b**.

Ongoing work is directed at investigating the synthesis of a library by modulating the Pro and Phe positions in β-turns.

Supporting Information Available: Experimental procedures and **1b** X-ray data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Analysis is based on HPLC peak areas at 214 and 254 nm of the linear hexapeptide and the cyclic compound.

(19) Crystal data: $C_{36}H_{42}N_6O_6 \cdot 5\ H_2O$, $M_t = 744.84$ g mol^{-1}, orthorhombic, space group $P2_12_12$, $a = 13.408(1)$, $b = 15.869(1)$, $c = 9.591(1)$ Å, $V = 2040.7(3)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.212$ Mg m^{-3}, Cu Kα radiation ($\lambda = 1.54178$ Å), $T = 293(2)$ K, $R_1 = 0.059$ [$I > 2\sigma(I)$] and w$R_2 = 0.17$ [$I > 2\sigma(I)$].

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