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Folding Types of Dipeptides Containing the Diastereoisomeric Cyclopropanic Analogues of Phenylalanine

Ana I. Jiménez*, Régis Vanderesse, Michel Marraud

Laboratoire de Chimie Physique Macromoléculaire, Unité de Recherche Associée au CNRS, ENSIC-INPL, BP 451, 54001 Nancy, France
Fax: (+33) 3 83 37 99 77; e-mail: jimenez@lcpm.ensic.u-nancy.fr

André Aubry

Laboratoire de Cristallographie et Modélisation des Matériaux Minéraux et Biologiques, Unité de Recherche Associée au CNRS, Université Henri Poincaré, BP 239, 54506 Vandœuvre, France
Fax: (+33) 3 83 40 64 92; e-mail: aubry@lcm3b.u-nancy.fr

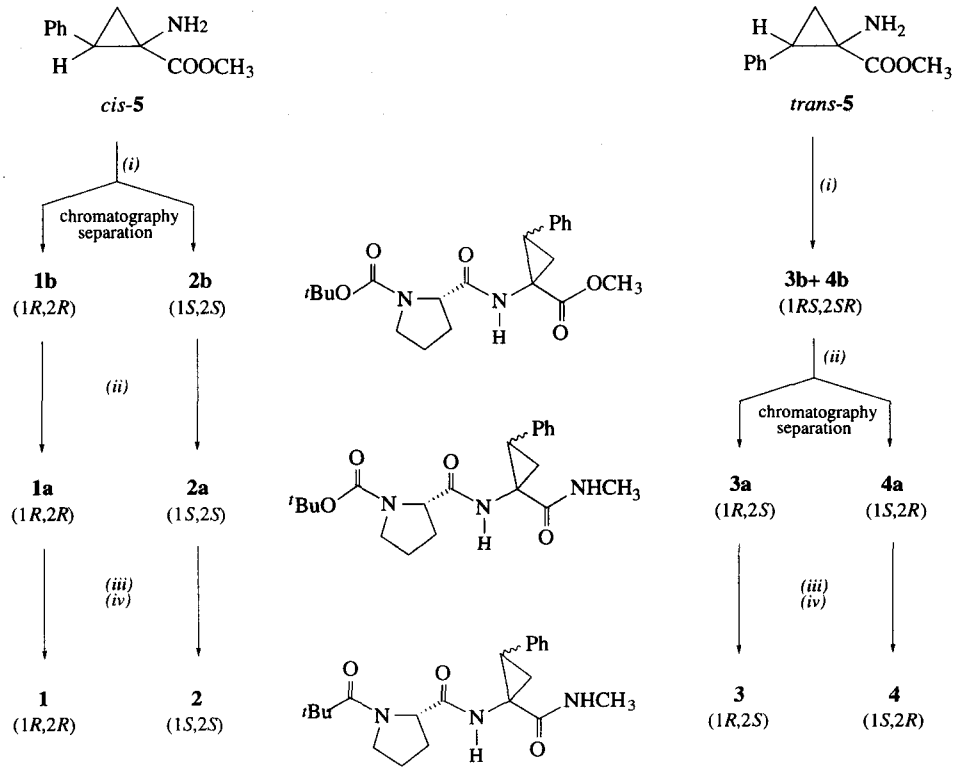
Carlos Cativiela

Departamento de Química Orgánica, Instituto de Ciencia de Materiales de Aragón, Universidad de Zaragoza-CSIC, 50009 Zaragoza, Spain
Fax: (+34) 9 76 76 20 77; e-mail: cativiela@posta.unizar.es

Abstract: In order to consider the possible influence of the orientation of a side chain on the peptide backbone, we have studied the molecular structure of four ^{t}BuCO-Pro-c_3Phe-NHMe model dipeptides, where c_3Phe denotes each of the four diastereoisomeric 2,3-methanophenylalanines, by using IR and proton NMR experiments. All four derivatives are β-folded, but the folding type depends on the stereochemistry of the cyclopropane moiety. © 1997 Published by Elsevier Science Ltd.

Conformations of peptides and proteins are mainly the consequence of backbone to backbone hydrogen bonds between amide groups, which induce particular orientations of the side substituents. Conversely, polar side substituents are capable of side chain-backbone interactions which may result in local structures.[1] Thus, orientation of the side chains not only could influence the conformation of the peptide chain, at least for steric reasons, but also could favour or inhibit molecular recognition processes which may require a particular orientation of the side substituents.[2] Some examples of rigid α-amino acid residues have given rise to potent hormone agonists or antagonists.[3]

α-Alkylation of amino acid residues is known to influence conformation of the peptide chains: the 3_{10} and α-helical structures are favoured by the α-amino isobutyric acid (Aib),[4] extended structures by open α,α-dialkylated residues,[4] and folded structures by 1-aminocycloalkanecarboxylic acid residues.[5,6] In the present study, we have taken advantage of the cyclopropane ring rigidity to study the folding mode of a peptide chain under the possible influence of the side chain orientation. To this end, we have considered the four ^{t}BuCO-Pro-c_3Phe-NHMe dipeptides **1-4** (Scheme 1), where c_3Phe denotes each of the four diastereoisomeric 2,3-methanophenylalanine residues. We have examined their *NH* and *CO* stretching frequencies in CH_2Cl_2, a weakly polar solvent allowing the existence of intramolecular hydrogen bonds. The solvent accessibility of the *NH* proton has been determined by proton NMR using $CDCl_3$/DMSO-d_6 mixtures with increasing content of DMSO-d_6, a strong NH solvating medium.[7]



Scheme 1.[8] (*i*) Boc-*L*-Pro-OH / ^{t}BuOCOCl / NMM / THF / -15°C; (*ii*) 8M $MeNH_2$ in MeOH; (*iii*) TFA / CH_2Cl_2; (*iv*) ^{t}BuCOCl / NMM / $CHCl_3$.

The racemic amino esters **5** were obtained by acid hydrolysis of their respective diphenylmethylene imines.[9] They were then coupled to N-*tert*-butyloxycarbonyl-*L*-proline by the classical mixed anhydride method using isobutyl chloroformate as coupling agent. The subsequent treatment with a methanolic solution of $MeNH_2$ afforded the corresponding methylamides in good yields. The *cis* diastereoisomeric esters **1b** and **2b**, and the *trans* diastereoisomeric amides **3a** and **4a** were separated by column chromatography. The Boc group in **1a-4a** was eliminated by treatment with trifluoroacetic acid, and the amino group was acylated by pivaloyl chloride. The advantage of the pivaloyl (Piv) group is to shift the carbonyl stretching to the 1600-1630 cm^{-1} domain, and to exclude the *cis* conformation of the Piv-Pro amide bond for sterical reasons.[7] The absolute configurations of the phenylalanine cyclopropanic residues in **1-4** were determined from the crystal molecular structures of **1** and **3a**, which were solved by X-ray diffraction.[10]

The vanishing absorption, if any, of the free *NH*(Me), at about 3450 cm^{-1}, indicated a negligible percentage of free sites in CH_2Cl_2 (Table 1). The low stretching frequency observed for this *NH* and the very weak sensitivity of this amide proton resonance in all four peptides **1-4**, when investigated in $CDCl_3$/DMSO-d_6 mixtures, indicated the participation of the NH(Me) group in a strong intramolecular hydrogen bond. On the contrary, the high frequency of the c_3Phe (**1-4**) *NH* stretching and the considerable sensitivity to DMSO-d_6 solvation of the *NH* resonance are typical of a free site.

Table 1. *NH* and *CO*(^{t}Bu) stretching frequencies in CH_2Cl_2 (ν, cm^{-1}; c = 5 mM)[a], *NH* proton resonance shift from $CDCl_3$ to DMSO-d_6 (Δδ, ppm; c = 10 mM) and nuclear Overhauser enhancement (NOE, %) of the Pro-$C^{\alpha}H$ resonance by irradiation of the c_3Phe-*NH* resonance ($CDCl_3$, c = 10 mM).

Peptide	c_3Phe	c_3Phe-*NH*		*NH*(Me)		*CO*(^{t}Bu)	NOE
		Δδ	ν	Δδ	ν	ν	
1	(1*R*,2*R*)	2.41	3408	0.03	3350	1602^{s} ; 1612^{vw}	28
2	(1*S*,2*S*)	3.04	3424 ; 3413	0.31	3360	1602^{m} ; 1611^{m}	17
3	(1*R*,2*S*)	2.02	3416	0.33	3362	1602^{s} ; 1614^{w}	23
4	(1*S*,2*R*)	2.21	3415	0.24	3358	1603^{s} ; 1613^{vw}	27

[a] Strong (s), medium (m), weak (w) or very weak (vw) absorption.

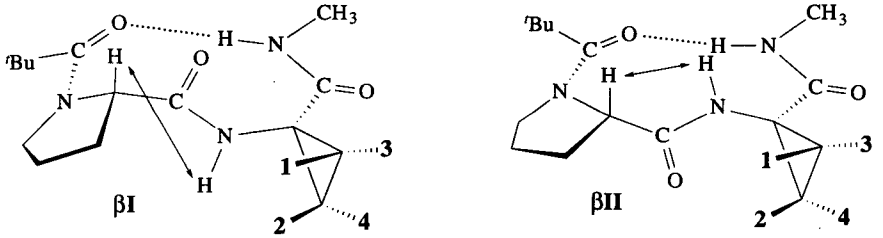


Figure 1. Schematic βI and βII-folding for ^{t}BuCO-Pro-c_3Phe-NHMe. Numbers **1-4** indicate the aromatic ring position for each peptide **1-4**. The arrows represent the NOE correlation between the two protons.

In CH_2Cl_2, the CO(^{t}Bu) carbonyl gave rise to two contributions at about 1602 and 1612 cm^{-1} with relative intensities depending on the c_3Phe chirality (Table 1). The lower component was the most intense for both **1**, **3** and **4**, and the upper one for **2**. Both were significantly shifted to low frequencies with reference to ^{t}BuCO-Pro-OMe (1619 cm^{-1}) where the CO(^{t}Bu) is free, and therefore corresponded to two different hydrogen bonded states of this carbonyl. It resulted that the NH(Me) and CO(^{t}Bu) sites were engaged in a hydrogen bond typical of a β-turn, but that the molecules accommodated two different folded conformations, their percentages depending on the orientation of the phenyl ring with reference to the folded peptide backbone.

Different families of β-folded structures have been characterized, but the so-called βI and βII-types, differing in the up or down orientation of the middle amide plane, are the most frequent (Figure 1).[11] The former is favoured for homochiral sequences, and the latter for heterochiral ones. It has been observed that they can be discriminated by the upper or lower stretching frequency of the hydrogen bonded carbonyl, or by the lower or upper Overhauser enhancement of the Pro-$C^{\alpha}H$ resonance by irradiation of the contiguous amide *NH* resonance.[7] In the present case, the values in Table 1 are in quite good agreement with the above statement. The

βII-turn is by far the most stable structure for both **1** and **4**, while significant amounts of the βI-turn are present for **3**, and even more for **2**.

To our knowledge, this is the first experimental evidence that the orientation of a side chain can induce a particular conformation of the peptide backbone. It offers the possibility to investigate the relationship between biological activity and side chain orientation in molecular recognition processes.[12,13] To this aim, modulation of the ring size and substituent nature in chiral 1-aminocycloalkanecarboxylic acids is in progress.

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