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Peptides from chiral $C^{\alpha\alpha}$-disubstituted glycines. On the helical screw sense of isovaline peptides

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Abstract. The preferred conformation of three N^{α}-acetylated Aib/Iva host/guest pentapeptide esters and their N^{α}-benzyloxycarbonylated synthetic precursors, prepared by solution methods and fully characterized, were examined in chloroform solution using FT-IR absorption and ^{1}H-NMR and in the crystal state by X-ray diffraction. All these peptides are folded in a 3_{10}-helix structure, irrespective of the experimental conditions used in the conformational analysis. In the crystal state the screw sense preference of the helical structure that is formed seems to be governed by the position of the single Iva residue in the peptide main chain, the ethyl side-chain disposition, and the nature of the N^{α}-blocking group.

Introduction

The stereochemistry of peptides containing $C^{\alpha,\alpha}$-disubstituted glycines is unique, as they possess significant constraints on their conformational freedom[1–3]. In particular, this property is relevant to the exploitation of these compounds as precise molecular rulers or templates in the *de-novo* design of protein mimetics[4,5], and as conformationally restricted, enzyme-resistant agonists and antagonists of bioactive peptides[6,7].

In this connection was found that homo-peptides from the prototypical achiral Aib (α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine) residue strongly prefer the 3_{10}-helical structure[2,3]. Recently, intriguing experimental findings on the impact of the chirality of (αMe)Val (C^{α}-methylvaline), (αMe)Leu (C^{α}-methylleucine), (αMe)Phe (C^{α}-methylphenylalanine), and (αMe)Trp (C^{α}-methyltryptophan) residues on 3_{10}-helix screw sense have been reported[2,8]. More specifically, (αMe)Val, with a *β-branched* side-chain, exhibits a *normal* behaviour, i.e. the same as that shown by protein amino acids [(*S*)-amino acids give *right*-handed helical structures], whereas the behaviour of (αMe)Leu, (αMe)Phe, and (αMe)Trp, with γ-branched side-chains, tends to be inverse with respect to that of protein amino acids.

The obvious next step along this line of research was to investigate the preferred conformation of Iva (isovaline or C^{α}-methyl-C^{α}-ethyl glycine)-containing peptides. Iva, the simplest chiral $C^{\alpha,\alpha}$-disubstituted glycine, has a linear side chain (ethyl group) and a methyl group linked to the α-carbon, and is a constituent of naturally occurring antibiotics where it is present either as the *R*- or, less frequently, as the *S*-enantiomer[9–11]. Conformational energy computations[12] and X-ray diffraction studies[13–18] of the few, scattered Iva peptides reported so far have failed to reveal a clear-cut bias toward either of the two possible helical screw senses.

In order to contribute to the solution of this problem, we synthesized, fully characterized, and examined in detail the solution and crystal-state conformational properties (using FT-IR absorption, ^{1}H NMR, and X-ray diffraction) of three N^{α}-acetylated Aib/Iva host/guest pentapeptide esters and their N^{α}-benzyloxycarbonylated synthetic precursors. The terminally blocked –(Aib)$_5$– host sequence is known to fold into a stable 3_{10}-helix[19–23]. In the host/guest pentapeptides the single Iva guest residue is located at positions 1, 2 and 4, all of them internal to the putative 3_{10}-helical structure. The X-ray diffraction structure of the N^{α}-acetylated pentapeptide ester with the Iva residue in the remaining internal position (position 3), published a few years ago[14], showed that the two crystallographic independent molecules in the asymmetric unit differ by the screw sense of their 3_{10}-helical structure. This was the first observation of a helical peptide containing a chiral residue without a preferred screw sense.

Results and discussion

Peptide synthesis

For the production of the enantiomerically pure (*S*)-Iva we exploited an economically attractive and generally applicable chemo-enzymatic synthesis developed by the DSM group a few years ago[24]. It involves a combination of organic syntheses for preparation of the racemic amino acid amide followed by the use of a broadly specific aminopeptidase to achieve optical resolution on a large scale.

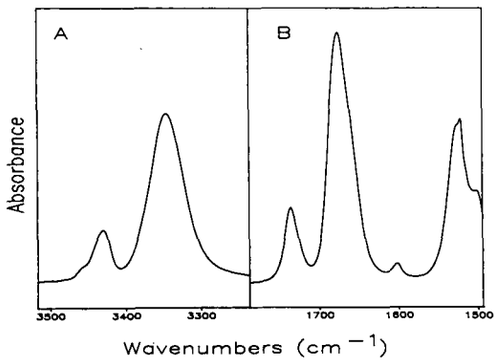

*Figure 1. FT-IR spectrum in the 3500–3250 cm^{-1} region (A) and in the 1750–1500 cm^{-1} region (B) of Ac-(Aib)$_3$-(S)-Iva-Aib-OMe (**3B**) in $CDCl_3$ solution (peptide concentration $1 \cdot 10^{-3}$ M).*

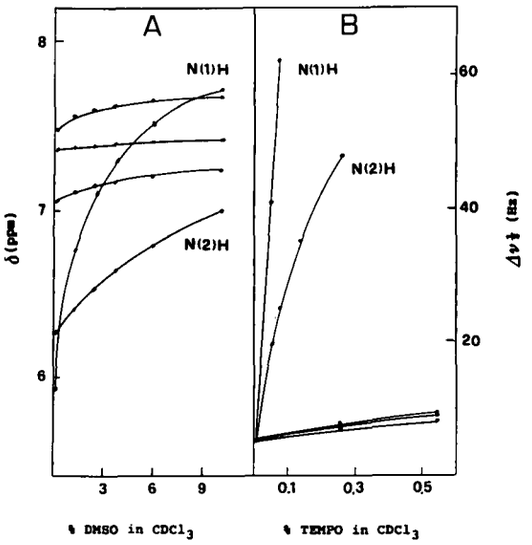

*Figure 2. (A) Plot of NH chemical shifts in the ^1H NMR spectrum of Ac-(Aib)$_3$-(S)-Iva-(Aib)-OMe (**3B**) as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). (B) Plot of the band width of the NH signals of the same peptide as a function of increasing percentages of TEMPO (w/v) in $CDCl_3$ solution (peptide concentration $1 \cdot 10^{-3}$ M).*

The synthesis and characterization of six terminally blocked Aib/(*S*)-Iva pentapeptides were performed. The compounds were characterized by thin-layer chromatography (TLC), polarimetry, solid-state IR absorption and ^1H NMR techniques.

The Iva-Aib coupling reactions were carried out in acetonitrile (MeCN) under reflux using the symmetrical anhydride of Z-(*S*)-Iva-OH[25]. Aib-Iva and (Aib)$_3$-Iva bond formation was achieved in MeCN under reflux using the symmetrical anhydride of Z-Aib-OH[26] and the 5(4*H*)-oxazolone from Z-(Aib)$_3$-OH[27], respectively.

Coupling yields optimization, e.g. by checking reaction time and excess of the carboxyl component, was not attempted. However, in general, a decrease in the coupling yield as a function of the main-chain length of the amino component was observed. This finding may be related to an enhancement of the stability of ordered secondary structure with an increase of the number of amino acid residues in the peptide chain. Removal of the Z group was performed by catalytic hydrogenation in methanol. N^{α}-Acetylation of the pentapeptide esters was obtained using acetic anhydride in MeCN.

Solution conformation

The preferred conformation of the three N^{α}-acetylated Aib/Iva host/guest pentapeptide esters was investigated in a structure-supporting solvent ($CDCl_3$) by FT-IR absorption and ^1H-NMR at various concentrations (over the range $1 \cdot 10^{-2} - 1 \cdot 10^{-4}$ M).

The FT-IR absorption spectrum of a representative pentapeptide, Ac-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**3**), in the 3500–3250 cm^{-1} and 1750–1500 cm^{-1} regions at $1 \cdot 10^{-3}$ M concentration is illustrated in Fig. 1 (the spectra of the other pentapeptides closely resemble that of the **3** analogue).

In the N–H stretching region (amide A) the spectral pattern is characterized by a weak band near 3425 cm^{-1} accompanied by shoulders at about 3440 and 3460 cm^{-1} (free, solvated NH groups), followed by an intense band at 3347 ± 1 cm^{-1} (strongly hydrogen-bonded NH groups)[28,29]. The C=O stretching mode of the methyl ester moiety is seen at 1737 cm^{-1}, while that of the amide and peptide groups (amide I) at 1678–1679 cm^{-1} (the latter accompanied by a shoulder at approximately 1660 cm^{-1}). The amide II band is located at 1522–1530 cm^{-1}. By recording the spectra at $1 \cdot 10^{-4}$ M in the N–H stretching region we have also been able to show that in the $1 \cdot 10^{-4} - 1 \cdot 10^{-3}$ M concentration range self-association via N–H...O=C intermolecular hydrogen bonding is negligible (less than 5%) for all pentapeptides (results not shown). Therefore, the remarkable hydrogen bonding exhibited by the pentapeptides should be interpreted as arising almost exclusively from intramolecular N–H...O=C interactions. In addition, the position of the intense amide A (near 3347 cm^{-1})[30,31] tends to exclude the onset of a significant amount of intramolecularly hydrogen-bonded extended (C_5) conformers[13,22], and to favour instead folded conformations of the β-bend (C_{10}) type[32–34].

To get additional information on the preferred folded conformation of the Aib/Iva pentapeptides in $CDCl_3$ solution, we carried out a 400-MHz ^1H-NMR study. The delineation of inaccessible (or intramolecularly hydrogen bonded) NH groups was performed using the solvent dependence of NH chemical shifts by adding increasing amounts of the strong, hydrogen-bonding acceptor DMSO[35,36] to the $CDCl_3$ solution, and free-radical (TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxy) induced line broadening of NH resonances[37].

In $CDCl_3$ solution a partial, tentative assignment was performed for the two upfield resonances (below 6.5 ppm) to the N(1)H and N(2) protons, by analogy with the chemical shifts in the same halohydrocarbon and the spectroscopic behaviour upon addition of perturbing agents of other N^{α}-acetylated homo-peptide esters from different types of $C^{\alpha,\alpha}$-disubstituted glycines[23,38,39].

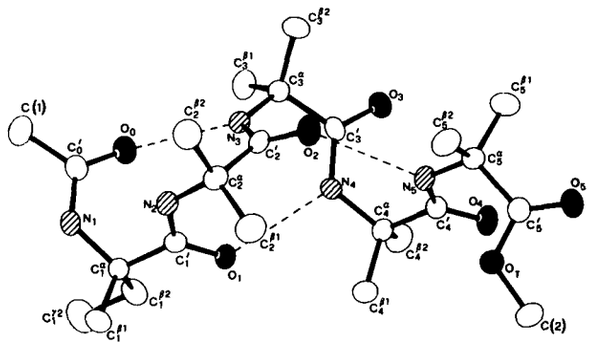

*Figure 3. X-ray diffraction structure of Ac-(S)-Iva-(Aib)$_4$-OMe (**1**) with atom numbering. The three intramolecular hydrogen bonds are represented as dashed lines.*

From an analysis of the spectra as a function of peptide concentration (between $1 \cdot 10^{-3}$ and $7 \cdot 10^{-3}$ M) in $CDCl_3$ solution (results not shown), we have been able to conclude that dilution induces only a negligible (≤ 0.02 ppm) upfield shift for all the NH resonances, except for the NH resonance at the highest field (5.71–5.95 ppm), whose shift is somewhat significant (0.03–0.10 ppm)[40]. In the three pentapeptides examined in the $CDCl_3$/DMSO solvent mixtures and in the presence of the paramagnetic perturbing agent TEMPO at 10^{-3} M peptide concentration (for one representative example see Fig. 2) two classes of NH protons were observed: (*i*) the first class [N(1)H and N(2)H protons] includes protons whose chemical shifts are sensitive to the addition of DMSO and whose resonances broaden significantly upon addition of TEMPO. Interestingly, the sensitivity of the N(1)H proton is higher than that of the N(2)H proton; (*ii*) the second class (the three other NH protons) includes those displaying a behaviour characteristic of shielded protons (relative insensitivity of chemical shifts to solvent composition, and of linewidths to the presence of TEMPO).

These ^{1}H-NMR results allow us to conclude that, in $CDCl_3$ solution below $7 \cdot 10^{-3}$ M concentration, the N^{α}-acetylated Aib/Iva pentapeptide esters have a modest propensity to self-associate and that in this process the amide N(1)H proton plays a major role as H-bonding donor. In the absence of self-association, the N(3)H–N(5)H protons are almost inaccessible to perturbing agents and are, therefore, most probably intramolecularly hydrogen-bonded. In view of these observations it is reasonable to suggest that the most populated structure adopted in $CDCl_3$ solution by these pentapeptides is the 3_{10}-helix (a series of three consecutive β-bend structures)[41]. These conclusions are in agreement with those extracted from the FT-IR absorption study discussed above.

Crystal-state conformation

We determined by X-ray diffraction the molecular and crystal structures of the following terminally blocked pentapeptides: Ac-(*S*)-Iva-(Aib)$_4$-OMe (**1**, Fig. 3), Ac-Aib-(*S*)-Iva-(Aib)$_3$-OMe (**2**, Fig. 4), Ac-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**3A,B**, Fig. 5), Z-(*S*)-Iva-(Aib)$_4$-OMe (**4**, Fig. 6), Z-Aib-(*S*)-Iva-(Aib)$_3$-OMe (**5**, Fig. 7), and Z-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**6**, Fig. 8). Two conformationally distinct, independent molecules (**A** and **B**) are present in the asymmetric unit of **3**. Relevant torsion angles are given in Table I. In Table II the intra- and intermolecular hydrogen-bond parameters are listed.

Bond lengths and bond angles are in general agreement with previously reported values for the geometry of acetamido[42] and benzyloxycarbonylamino[43] moieties, the

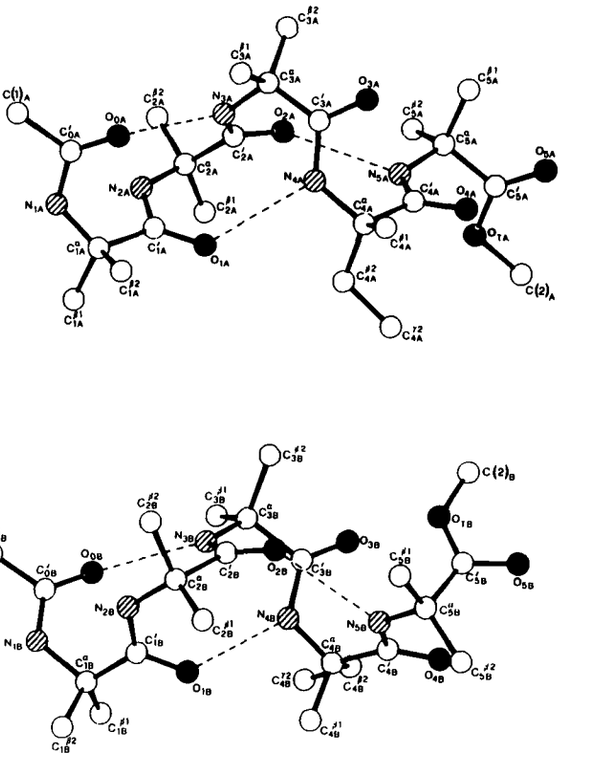

*Figure 5. X-ray diffraction structure of the two independent molecules A and B in the asymmetric unit of Ac-(Aib)$_3$-(S)-Iva-Aib-OMe (**3**) with atom numbering. In each molecule the three intramolecular hydrogen bonds are represented as dashed lines.*

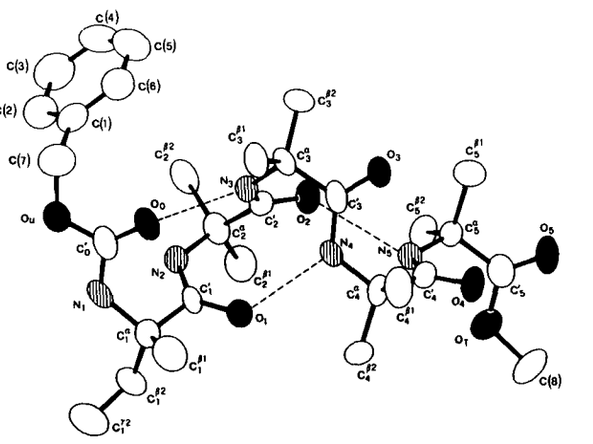

*Figure 6. X-ray diffraction structure of Z-(S)-Iva-(Aib)$_4$-OMe (**4**) with atom numbering. The three intramolecular hydrogen bonds are represented as dashed lines.*

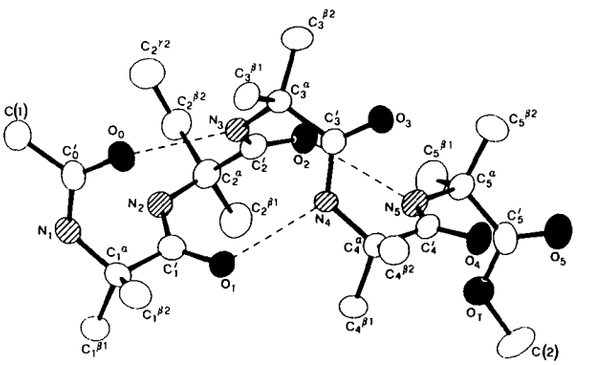

*Figure 4. X-ray diffraction structure of Ac-Aib-(S)-Iva-(Aib)$_3$-OMe (**2**) with atom numbering. The three intramolecular hydrogen bonds are represented as dashed lines.*

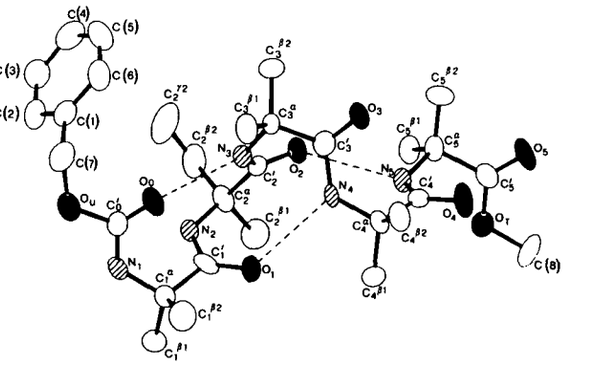

*Figure 7. X-ray diffraction structure of Z-Aib-(S)-Iva-(Aib)$_3$-OMe (**5**) with atom numbering. The three intramolecular hydrogen bonds are represented as dashed lines.*

Table I Selected torsion angles (°) for the six Aib / Iva host / guest pentapeptides.

Torsion angle	Ac-(*S*)-Iva-(Aib)$_4$-OMe (**1**)	Ac-Aib-(*S*)-Iva-(Aib)$_3$-OMe (**2**)	Ac-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**3**)		Z-(*S*)-Iva-(Aib)$_4$-OMe (**4**)	Z-Aib-(*S*)-Iva-(Aib)$_3$-OMe (**5**)	Z-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**6**)
			Mol. **A**	Mol. **B**			
θ^3	–	–	–	–	60.6(8)	55.0(12)	71.8(7)
θ^2	–	–	–	–	82.5(8)	86.3(8)	86.1(6)
θ^1	–	–	–	–	173.1(6)	175.6(6)	174.8(5)
ω_0	171.6(7)	−176.7(6)	−172.6(13)	173.7(12)	−179.1(6)	−173.0(7)	−178.3(5)
ϕ_1	56.3(10)	−59.5(8)	−54.0(18)	53.1(17)	−55.9(8)	−55.8(10)	−59.3(6)
ψ_1	36.6(10)	−28.5(8)	−35.7(18)	33.6(17)	−31.2(8)	−35.1(10)	−27.2(7)
ω_1	175.4(7)	179.8(6)	−176.1(12)	172.9(12)	−177.2(5)	−175.4(7)	−179.4(5)
ϕ_2	59.4(10)	−52.4(8)	−55.6(17)	58.5(17)	−51.8(8)	−56.3(9)	−54.8(7)
ψ_2	19.1(10)	−27.7(8)	−26.8(17)	22.2(16)	−32.4(8)	−22.6(9)	−27.9(7)
ω_2	−175.0(7)	−178.2(6)	−177.1(12)	−179.3(11)	−176.3(5)	−177.9(6)	−178.1(5)
ϕ_3	54.3(10)	−57.1(8)	−56.7(16)	54.2(16)	−53.1(8)	−54.8(9)	−54.2(7)
ψ_3	24.7(10)	−27.8(8)	−24.0(17)	32.6(16)	−32.9(8)	−30.6(9)	−27.1(7)
ω_3	−176.1(7)	179.2(5)	172.8(12)	172.8(11)	−177.0(5)	−176.4(7)	178.9(5)
ϕ_4	50.8(10)	−54.1(7)	−43.6(17)	55.1(17)	−56.4(8)	−56.5(9)	−51.7(6)
ψ_4	32.9(10)	−35.4(8)	−39.7(17)	33.1(17)	−33.3(8)	−32.8(9)	−39.4(6)
ω_4	177.2(7)	−177.7(5)	173.6(13)	176.1(13)	171.0(5)	169.0(7)	178.7(4)
ϕ_5	−47.8(10)	48.0(8)	52.8(19)	63.9(18)	46.5(8)	48.1(9)	50.2(6)
ψ_5	−44.4(9)	47.4(8)	54.0(18)	61.6(19)	55.0(7)	55.1(9)	47.7(7)
ω_5	176.5(7)	178.3(6)	171.1(14)	179.2(16)	174.5(6)	173.7(7)	179.4(5)
χ^{12}	−53.7(9)	63.0(8)	169.1(13)	−57.7(18)	−66.0(8)	64.8(9)	172.5(4)

ester group[44], the peptide unit[45,46], and the Aib[47,48] and Iva[13–18] residues.

All the six pentapeptides, including both molecules **A** and **B** of the **3** analogue, are folded in a regular 3_{10}-helix with three consecutive $1 \leftarrow 4$ C=O...H–N intramolecular hydrogen bonds. The $N_3 \cdots O_0$, $N_4 \cdots O_1$, and $N_5 \cdots O_2$ intramolecular separations are in the expected range [2.910(13) – 3.085(6) Å][49–51]. The average absolute values for the ϕ, ψ torsion angles of all residues internal to the 3_{10}-helical structures (positions 1–4) are 55, 30°, close to those expected for a regular 3_{10}-helix (57, 30°)[41]. As for the screw sense of the 3_{10}-helices that are formed, those of **2**, **3A**, and all three N^{α}-Z pentapeptides are right-handed, while those of **1** and **3B** are left-handed. Also the C-terminal Aib residues of all the pentapeptide molecules are helical but, with the only exception of **3B** they show an opposite screw sense with respect to those of the preceding residues, a common observation for 3_{10}-helix forming peptide esters[52,53].

The distribution of the ethyl side-chain χ^1 torsion angles for the seven (*S*)-Iva residues is $2g^+$ (*gauche*$^+$), $3g^-$ (*gauche*$^-$), and $2t$ (*trans*). More specifically, the two t and the two g^+ conformations are adopted by (*S*)-Iva residues in the right-handed helical conformation, while in the two (*S*)-Iva residues in the left-handed helical conformation the g^- side-chain orientation is observed. The only exception to this generalization is seen in the right-handed helical **4** analogue with a g^- side chain. The

Table II Intra- and intermolecular hydrogen bond parameters for the six Aib / Iva host / guest peptides.

Peptide	Donor D-H	Acceptor A	Symmetry operations of A	Distance (Å)		Angle (°) D-H....A
				D...A	H...A	
Ac-(*S*)-Iva-(Aib)$_4$-OMe (**1**)	N_3-H	O_0	*x,y,z*	3.012(6)	1.971(3)	157.5(7)
	N_4-H	O_1	*x,y,z*	2.958(7)	1.897(5)	167.9(3)
	N_5-H	O_2	*x,y,z*	2.947(6)	1.920(4)	159.0(3)
	N_1-H	O_4	*x,1 + y,z*	2.806(6)	1.788(4)	156.9(7)
Ac-Aib-(*S*)-Iva-(Aib)$_3$-OMe (**2**)	N_3-H	O_0	*x,y,z*	2.989(8)	1.923(5)	164.8(3)
	N_4-H	O_1	*x,y,z*	2.953(6)	1.894(4)	160.2(3)
	N_5-H	O_2	*x,y,z*	2.976(6)	2.042(4)	162.6(3)
	N_1-H	O_4	*1 + x,y,z*	2.807(6)	1.788(5)	151.1(3)
Ac-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**3**)	N_{3A}-H	O_{0A}	*x,y,z*	3.018(12)	1.980(12)	160.1(10)
	N_{4A}-H	O_{1A}	*x,y,z*	3.031(12)	1.990(11)	160.7(10)
	N_{5A}-H	O_{2A}	*x,y,z*	2.910(13)	1.930(13)	149.1(10)
	N_{3B}-H	O_{0B}	*x,y,z*	3.038(13)	1.997(13)	161.0(11)
	N_{4B}-H	O_{1B}	*x,y,z*	2.976(12)	1.936(11)	160.7(11)
	N_{5B}-H	O_{2B}	*x,y,z*	3.027(14)	2.039(14)	150.6(13)
	N_{1A}-H	O_{4A}	*x-1,y,z*	2.820(11)	1.793(11)	157.1(11)
	N_{1B}-H	O_{4B}	*x-1,y,z*	2.793(12)	1.724(12)	169.7(11)
	N_{2A}-H	O_{5A}	*x-1,y,z*	3.262(13)	2.445(13)	131.4(9)
	N_{2B}-H	O_{5B}	*x-1,y,z*	3.222(15)	2.235(14)	151.1(10)
Z-(*S*)-Iva-(Aib)$_4$-OMe (**4**)	N_3-H	O_0	*x,y,z*	3.054(6)	2.026(6)	157.8(4)
	N_4-H	O_1	*x,y,z*	3.005(6)	1.984(6)	156.5(6)
	N_5-H	O_2	*x,y,z*	3.025(5)	2.151(6)	136.3(4)
	N_1-H	O_4	*x,y,1 + z*	2.817(6)	1.830(6)	150.0(6)
Z-Aib-(*S*)-Iva-(Aib)$_3$-OMe (**5**)	N_3-H	O_0	*x,y,z*	3.074(7)	2.024(7)	163.2(6)
	N_4-H	O_1	*x,y,z*	3.085(6)	2.027(6)	165.8(6)
	N_5-H	O_2	*x,y,z*	2.986(6)	2.090(6)	138.5(5)
	N_1-H	O_4	*x,y,1 + z*	2.789(7)	1.770(7)	155.5(7)
Z-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**6**)	N_3-H	O_0	*x,y,z*	3.031(5)	1.989(5)	161.1(4)
	N_4-H	O_1	*x,y,z*	3.037(6)	1.985(6)	163.8(4)
	N_5-H	O_2	*x,y,z*	2.964(6)	2.041(6)	141.7(4)
	N_1-H	O_4	*x,1 + y,z*	2.795(5)	1.932(5)	134.3(4)

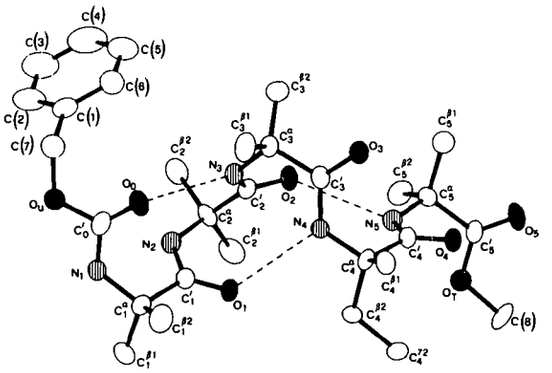

*Figure 8. X-ray diffraction structure of Z-(Aib)$_3$-(S)-Iva-Aib-OMe (**6**) with atom numbering. The three intramolecular hydrogen bonds are represented as dashed lines.*

two molecules **3A** and **3B** exhibit different side-chain orientations (*t* and g^-, respectively) in the asymmetric unit.

The acetamido, benzyloxycarbonylamino, peptide, and ester moieties are all in the common trans disposition, with only the ω_4 torsion angle of **5** and **6** deviating $\geq 10°$ from the ideal, planar 180° value. In addition to the ω_0 torsion angle, the conformation of the urethane group of the benzyloxycarbonylamino moiety is described by the θ^1 [N_1-C'_o-O_u-C(7)] torsion angle. The ω_0 and θ^1 torsion angles allow us to classify the urethane group of the three pentapeptides as the common type *b* (or *trans,trans*)[43]. The values for the θ^2 [C'_o-O_u-C(7)-C(1)] and θ^3 [O_u-C(7)-C(1)-C(2) or O_u-C(7)-C(1)-C(6)] torsion angles of the Z group are close to 90° and 60°, respectively. In all the pentapeptide molecules, the methyl ester disposition with respect to the C^α_5-N_5 bond is intermediate between the antiperiplanar and anticlinal conformations[54].

In the crystals of all the pentapeptides, rows of molecules are generated through (amide or urethane) N1-H...O_4 = C'_4 (peptide) intermolecular hydrogen bonds, along the *x*-axis for **2** and **3**, the *y*-axis for **1** and **6**, and the *z*-axis for **4** and **5**. The N_1...O_4 distances are in the range 2.789(7)–2.820(11) Å[49–51]. In the packing mode of molecules **A** and **B** of **3**, additional, weak intermolecular (peptide) N_{2A}-H...O_{5A} = C'_{5A} (ester) and (peptide) N_{2B}-H...O_{5B} = C'_{5B} (ester) hydrogen bonds are observed along the *x*-axis. The N_2...O_5 distances are 3.262(13) and 3.222(15) Å, respectively.

Conclusions

The results of this solution and crystal-state structural analysis of six terminally blocked Aib/Iva host/guest pentapeptides, together with those already published of Ac-(Aib)$_2$-(*S*)-Iva-(Aib)$_2$-OMe[14], strongly support the view that an Iva guest residue can be easily accomodated into the stable, regular 3_{10}-helical structure adopted by the Aib homo-peptide host sequence[19–23].

However, the most intriguing conclusion that can be extracted from all these crystal-state studies concerns the relationship between Iva α-carbon chirality and helix screw sense. Since Aib is achiral, the screw sense of the helices of these peptides is dictated exclusively by the α-carbon configuration of the single guest (*S*)-Iva residue. In six out of the nine molecules we find a *normal* relationship, typical of protein amino acids, whereas in three molecules an *inverse* relationship is observed. Interestingly, all the three N^α-benzyloxycarbonylated pentapeptide esters exhibit *normal* behaviour. Taken together, all these findings point to a small difference in energy between the two diastereomeric 3_{10}-helical conformations[12,14,15], but to a non-negligible effect induced by (*i*) nature of the N^α-blocking group, (*ii*) position of the single chiral Iva residue in the helical peptide main-chain, and (*iii*) ethyl side-chain disposition.

In summary, the information available so far seems to suggest that C^α-methylated amino acids with a *linear* side chain (as in Iva) of (*S*) chirality would favour a right-handed 3_{10}-helical structure. However, it is reasonable to assume that the definitive solution of this problem might

Table III Crystal data for the six Aib / Iva host / guest pentapeptides.

Parameter	Ac-(*S*)-Iva-(Aib)$_4$-OMe (**1**)	Ac-Aib-(*S*)-Iva-(Aib)$_3$-OMe (**2**)	Ac-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**3**)	Z-(*S*)-Iva-(Aib)$_4$-OMe (**4**)	Z-Aib-(*S*)-Iva-(Aib)$_3$-OMe (**5**)	Z-(Aib)$_3$-(*S*)-Iva-Aib-OMe (**6**)
Mol. formula	$C_{24}H_{43}N_5O_7$	$C_{24}H_{43}N_5O_7$	$C_{24}H_{43}N_5O_7$	$C_{30}H_{47}N_5O_8$	$C_{30}H_{47}N_5O_8$	$C_{30}H_{47}N_5O_8$
MW (a.m.u.)	513.6	513.6	513.6	605.7	605.7	605.7
Crystal size (mm)	0.4x0.4x0.4	0.2x0.4x0.8	0.15x0.15x0.2	0.14x0.24x0.5	0.12x0.12x0.3	0.2x0.2x0.2
Crystal system	triclinic	monoclinic	monoclinic	monoclinic	monoclinic	monoclinic
Space group	*P*1	$P2_1$	$P2_1$	$P2_1$	$P2_1$	$P2_10$
Z (mol./unit cell)	1	2	4	2	2	2
a (Å)	8.417(1)	11.492(2)	11.842(2)	8.839(1)	8.928(1)	17.199(2)
b (Å)	11.758(2)	8.408(1)	17.832(2)	16.675(2)	16.115(2)	11.332(2)
c (Å)	8.211(1)	15.999(2)	14.315(2)	11.521(2)	11.706(2)	8.655(2)
α (°)	90.7(2)	90.0	90.0	90.0	90.0	90.0
β (°)	116.2(2)	107.5(2)	106.1(2)	90.4(1)	91.2(1)	90.0(1)
γ (°)	85.5(2)	90.0	90.0	90.0	90.0	90.0
V (Å^3)	727(1)	1474(2)	2904(3)	1698(1)	1684(1)	1687(1)
Density (calcd.) g/cm^3	1.174	1.1570	1.175	1.185	1.195	1.193
Indep. reflections	3507	1352	2337	2326	3995	1387
Observed reflections	1492	1335	1608	1644	1136	1287
	$[F \geq 6\sigma(F)]$	$[F \geq 2\sigma(F)]$	$[F \geq 4\sigma(F)]$	$[F \geq 3\sigma(F)]$	$[F \geq 2\sigma(F)]$	$[F \geq 6\sigma(F)]$
R value	0.030	0.040	0.074	0.048	0.045	0.050
R_w value	0.031	0.041	0.071	0.047	0.029	0.047
w	$1/[\sigma^2(F)+0.0013F^2]$	$1/[\sigma^2(F)+0.00277F^2]$	$1/[\sigma^2(F)+0.0068F^2]$	$1/[\sigma^2(F)+0.0029F^2]$	$1/[\sigma^2(F)]$	$1/[\sigma^2(F)+0.0134F^2]$
F(000)	278	556	1112	652	652	652
μ(mm^{-1})	0.081	0.080	0.68	0.081	0.081	0.68
S	0.65	0.65	1.07	0.73	0.55	0.53
$(\Delta\rho)_{max}$ (e/Å^3)	0.10	0.13	0.28	0.18	0.15	0.19
$(\Delta\rho)_{min}$ (e/Å^3)	−0.11	−0.15	−0.28	−0.24	−0.16	−0.24
$(\Delta/\sigma)_{max}$	0.74	0.33	0.22	0.55	0.23	0.37

be obtained from the conformational investigation of homo-chiral Iva homo-peptides currently in progress in our laboratories.

Experimental

Infrared absorption

Infrared (IR) absorption spectra were recorded with a Perkin-Elmer (Norwalk, CT) model 1720X FT-IR spectrometer, nitrogen flushed, at 2 cm^{-1} nominal resolution, averaging 64 scans for 10 mM sample concentration, or 100 scans for 1.0 and 0.1 mM sample concentrations. Solvent (base line) spectra were obtained under the same conditions. Cells with path length 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde deuteriochloroform (99.8% ^2H) was purchased from Merck. For the solid-state measurements the KBr disk technique was used.

^1H-NMR

The ^1H-NMR spectra were recorded either with a Bruker (Karlsruhe, Germany) model WP 200 SY or with a Bruker model AM 400 spectrometer. Measurements were carried out in deuteriochloroform (99.96% ^2H; Aldrich, Milwaukee, WI) and in DMSO (99.96% d_6; Fluka, Büchs, Switzerland) with tetramethylsilane as the internal standard. The free radical TEMPO was purchased from Sigma (St. Louis, MO).

X-ray diffraction

Colourless crystals of **1–6** were grown from methanol/water (slow evaporation), acetone/petroleum ether (vapour diffusion), methanol (slow evaporation), acetone/petroleum ether (vapour diffusion), methanol (slow evaporation), and methanol (slow evaporation), respectively. Reflections were collected on a Philips PW 1100 diffractometer (Eindhoven, The Netherlands), using the θ-2θ scan mode to θ 28° and graphite-monochromated MoKα radiation (λ 0.7107 Å) for all the pentapeptides, except for **3** and **6** whose reflections were collected to θ 44° by using graphite-monochromated CuKα radiation (λ 1.5418 Å). The structures of **1**, **4** and **5** were solved by direct methods using the SHELXS 86 program[56], and refined by full-matrix blocked least-squares with all non-hydrogen atoms anisotropic using the SHELX 76 program[57]. The structures of **2** and **6** were solved by the PATSEE program[58] using a search fragment taken from the structure of **1**. A structure expansion with the tangent formula as in SHELXS-86 revealed the position of the remaining non-hydrogen atoms. As for **3**, application of the PATSEE program allowed the location of the non-hydrogen atoms of molecule **A**. Structure expansion revealed most of the non-hydrogen atoms of molecule **B**. The remaining non-hydrogen atoms of molecule **B** were located on successive ΔF maps. Refinements were carried out as described above for the other pentapeptides. The hydrogen atoms of **1** and **2** were in part located on a ΔF map and in part calculated, and they were not refined. The hydrogen atoms of **3** were calculated and treated in the "riding mode" with U_{iso} fixed. The poor quality of the crystals of this pentapeptide accounts for the low number of observed reflections which, in turn, is responsible for the significant deviation of some geometrical parameters from the ideal values, for the large e.s.d.'s of atomic coordinates and thermal parameters, and for the large R_{max}/R_{min} ratios of thermal ellipsoids of many atoms. Nevertheless, we believe that the molecular conformation is safely established. The hydrogen atoms on the phenyl ring of the three N^{α}-Z pentapeptides were calculated and not refined. The remaining hydrogen atoms were in part located on a ΔF map, in part calculated, and they were treated in the "riding mode" with U_{iso} fixed. Table III gives the crystal data for the six Aib/Iva host/guest pentapeptides. Complete lists of bond lengths, bond angles, and torsion angles, the final positional parameters for all non-hydrogen atoms along with their thermal factors have been deposited and are available from the Cambridge Crystallographic Data Centre.

Synthesis of peptides

Melting points were determined on a Leitz model Laborlux 12 apparatus (Wetzler, Germany) and are uncorrected. Polarimetric measurements were performed on a Perkin-Elmer model 241 polarimeter equipped with a Haake model L thermostat (Karlsruhe, Germany). TLC was carried out on silica-gel plates 60F-254 (Merck, Darmstadt, Germany), using the following solvent systems: (I) chloroform/ethanol 9/1; (II) 1-butanol/acetic acid/water 6/2/2; (III) toluene/ethanol 7/1. The compounds were revealed either with the aid of a UV lamp or using the hypochlorite-starch-iodide chromatic reaction. A single spot was observed in each case.

Z-(S)-Iva-Aib-OMe was prepared from [Z-(*S*)-Iva]$_2$O[25] and H-Aib-OMe (obtained from HCl·H-Aib-OMe and *N*-methylmorpholine) in anhydrous MeCN at room temperature for 72 h and under reflux for 5 h. A large excess (50%) of symmetrical anhydride was used. The solvent was removed in vacuo and the residue taken up in AcOEt. The organic layer was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$, and water, dried over Na_2SO_4, filtered and evaporated to dryness; yield 98%; m.p. 101–102°C (from AcOEt/petroleum-ether); TLC R_F(I) 0.80, R_F(II) 0.90, R_F(III) 0.45; $[\alpha]_D^{20}$ 1.7° (*c* 0.5, MeOH); $[\alpha]_{436}^{20}$ 3.7° (*c* 0.5, MeOH). IR (KBr): 3409, 3305, 1730, 1719, 1660, 1532 cm^{-1}. ^{1}H-NMR ($CDCl_3$, $1 \cdot 10^{-2}$ M): δ 7.35, m, 5H, Z phenyl CH; 6.69, s, 1H, Aib NH; 5.58, s, 1H, Iva NH; 5.09, m, 2H, Z CH_2; 3.73, s, 3H, OMe CH_3; 2.12 and 1.71, 2m, 2H, Iva β-CH_2; 1.52, m, 9H, Aib and Iva β-CH_3; 0.84, m, 3H, Iva γ-CH_3.

Z-(Aib)$_3$-(S)-Iva-Aib-OMe (**6**) was synthesized from the reaction of the 5(4*H*)-oxazolone from Z-(Aib)$_3$-OH[27] and H-(*S*)-Iva-Aib-OMe (the latter obtained by catalytic hydrogenation in MeOH of the corresponding Z derivative) in anhydrous MeCN under reflux for 11 h. Part of the product precipitated out of the reaction mixture upon cooling to room temperature. This solid material was filtered off. The work-up procedure of the organic solution is the same as that described above for Z-(*S*)-Iva-Aib-OMe; total yield 77%; m.p. 221–223°C (from MeOH/diethyl-ether); TLC R_F(I) 0.60, R_F(II) 0.85, R_F(III) 0.20; $[\alpha]_D^{20}$ −2.3° (c 0.5, MeOH); $[\alpha]_{436}^{20}$ −6.1° (*c* 0.5, MeOH). IR (KBr): 3412, 3328, 3308, 3249, 1741, 1701, 1654, 1537 cm^{-1}. ^{1}H-NMR ($CDCl_3$, $1 \cdot 10^{-2}$ M): δ 7.41, s, 1H, NH; 7.37, m, 5H, Z phenyl CH; 7.36, s, 1H, NH; 7.04, s, 1H, NH; 6.28, s, 1H, NH; 5.21, s, 1H, Aib^1 NH; 5.12, m, 2H, Z CH_2; 3.67, s, 3H, OMe CH_3; 2.12 and 1.79, 2m, 2H, Iva β-CH_2; 1.46 and 1.30, 2m, 27H, Aib and Iva β-CH_3; 0.86, m, 3H, Iva γ-CH_3.

Ac-(Aib)$_3$-(S)-Iva-Aib-OMe (**3**) was prepared from the reaction of H-(Aib)$_3$-(*S*)-Iva-Aib-OMe (obtained by catalytic hydrogenation in MeOH of the corresponding Z derivative) and acetic anhydride in anhydrous MeCN at room temperature for 22 h. The work-up procedure was the same as that described above for Z-(*S*)-Iva-Aib-OMe; yield 89%; m.p. 282–284°C (from MeOH/diethyl-ether); R_F(I) 0.20, R_F(II) 0.65, R_F(III) 0.05; $[\alpha]_D^{20}$ −6.0° (*c* = 0.5, MeOH); $[\alpha]_{436}^{20}$ −14.9° (*c* = 0.5, MeOH). IR (KBr): 3404, 3296, 1733, 1662, 1539 cm^{-1}. ^{1}H-NMR ($CDCl_3$, $5 \cdot 10^{-3}$ M): δ 7.52, s, 1H, NH; 7.39, s, 1H, NH; 7.09, s, 1H, NH; 6.30, s, 1H, NH; 5.98, s, 1H, NH; 3.68, s, 3H, OMe CH_3; 2.10 and 1.80, 2m, 2H, Iva β-CH_2; 2.06, s, 3H, Ac CH_3; 1.48, m, 27H, Aib and Iva β-CH_3; 0.85, m, 3H, Iva γ-CH_3.

Z-(S)-Iva-(Aib)$_3$-OMe was prepared from the reaction of [Z-(*S*)-Iva]$_2$O and H-(Aib)$_3$-OMe [the latter obtained by catalytic hydrogenation in MeOH of Z-(Aib)$_3$-OMe[55]] in anhydrous MeCN under reflux for 5 h. The work-up procedure was the same as that described above for Z-(*S*)-Iva-Aib-OMe; yield 88%; m.p. 170–171°C (from AcOEt/petroleum-ether); R_F(I) 0.60, R_F(II) 0.85, R_F(III) 0.25; $[\alpha]_D^{20}$ 5.5° (*c* 0.5, MeOH); $[\alpha]_{436}^{20}$ 7.6° (*c* 0.5, MeOH). IR (KBr): 3366, 3330, 1728, 1701, 1656, 1534 cm^{-1}. ^{1}H-NMR ($CDCl_3$, $1 \cdot 10^{-2}$ M): δ 7.37, m, 5H, Z phenyl CH; 7.31, s, 1H, Aib NH; 7.13, s, 1H, Aib NH; 6.29, s, 1H, Aib NH; 5.12, m, 2H, Z CH_2; 5.10, s, 1H, Iva NH; 3.69, s, 3H, OMe CH_3; 1.80 and 1.51, 2m, 2H, Iva β-CH_2; 1.40, m, 21H, Aib and Iva β-CH_3; 0.90, m, 3H, Iva γ-CH_3.

Z-Aib-(S)-Iva-(Aib)$_3$-OMe (**5**) was synthesized from the reaction of (Z-Aib)$_2$O[26] and H-(*S*)-Iva-(Aib)$_3$-OMe (the latter obtained by catalytic hydrogenation in MeOH of the corresponding Z derivative) in anhydrous MeCN under reflux for 25 h. The work-up procedure was the same as that described above for Z-(*S*)-Iva-Aib-OMe; yield: 77%; m.p. 213–215°C (from AcOEt); R_F(I) 0.55, R_F(II) 0.90, R_F(III) 0.20; $[\alpha]_D^{20}$ 5.3° (*c* 0.5, MeOH); $[\alpha]_{436}^{20}$ 9.2° (*c* 0.5, MeOH). IR (KBr): 3399, 3332, 3301, 3258, 1730, 1700, 1668, 1646, 1535 cm^{-1}. ^{1}H-NMR ($CDCl_3$, $5 \cdot 10^{-3}$ M): δ 7.41, s, 1H, NH; 7.39, s, 1H, NH; 7.36, m, 5H, Z phenyl CH; 7.23, s, 1H, NH; 6.26, s, 1H, NH; 5.21, s, 1H, Aib^1 NH; 5.11, m, 2H, Z CH_2; 3.68, s, 3H, OMe CH_3; 1.80 and 1.55, 2m, 2H, Iva β-CH_2; 1.48, m, 24H, Aib/Iva β-CH_3; 1.32, s, 3H, Aib/Iva β-CH_3; 0.72, m, 3H, Iva γ-CH_3.

Ac-Aib-(S)-Iva-(Aib)$_3$-OMe (**2**) was prepared from the reaction of H-Aib-(*S*)-Iva-(Aib)$_3$-OMe (obtained by catalytic hydrogenation in MeOH of the corresponding Z derivative) and acetic anhydride in anhydrous MeCN at room temperature for 22 h. The work-up

procedure was the same as that described above for Z-(*S*)-Iva-Aib-OMe; yield 94%; m.p. 281–283°C (from MeOH/diethyl-ether); R_F(I) 0.25, R_F(II) 0.70, R_F(III) 0.05; $[\alpha]_D^{20}$ 7.6° (*c* 0.5, MeOH); $[\alpha]_{436}^{20}$ 11.3° (*c* 0.5, MeOH). IR (KBr): 3426, 3315, 3272, 1734, 1665, 1643, 1537 cm^{-1}. ^{1}H-NMR ($CDCl_3$, $5 \cdot 10^{-3}$ M): δ 7.53, s, 1H, NH; 7.43, s, 1H, NH; 7.28, s, 1H, NH; 6.27, s, 1H, NH; 6.00, s, 1H, NH; 3.69, s, 3H, OMe CH_3; 2.07, s, 3H, Ac CH_3; 1.92 and 1.69, 2m, 2H, Iva β-CH_2; 1.48, m, 27H, Aib and Iva β-CH_3; 0.86, m, 3H, Iva γ-CH_3.

Z-(S)-Iva-(Aib)$_4$-OMe (**4**) was synthesized from the reaction of [Z-(*S*)-Iva]$_2$O and H-(Aib)$_4$-OMe (the latter obtained by catalytic hydrogenation in MeOH of Z-(Aib)$_4$-OMe[23]) in anhydrous MeCN at room temperature for 32 h and under reflux for 4 h. A slight excess (5%) of symmetrical anhydride was used. The work-up procedure was the same as that described above for Z-(*S*)-Iva-Aib-OMe; yield 49%; m.p. 248–249°C (from AcOEt); R_F(I) 0.75, R_F(II) 0.95, R_F(III) 0.20; $[\alpha]_D^{20}$ 4.4° (*c* 0.5, MeOH); $[\alpha]_{436}^{20}$ 8.5° (*c* 0.5, MeOH). IR (KBr): 3412, 3322, 3260, 1732, 1701, 1651, 1533 cm^{-1}. ^{1}H-NMR ($CDCl_3$, $5 \cdot 10^{-3}$ M): δ 7.40, s, 1H, NH; 7.37, m, 5H, Z phenyl CH; 7.22, s, 2H, 2NH; 6.26, s, 1H, NH; 5.11, m, 2H, Z CH_2; 5.09, s, 1H, Iva NH; 3.69, s, 3H, OMe CH_3; 1.79, m, 2H, Iva β-CH_2; 1.56-1.27, m, 27H, Aib and Iva β-CH_3; 0.91, m, 3H, Iva γ-CH_3.

Ac-(S)-Iva-(Aib)$_4$-OMe (**1**) was prepared from the reaction of H-(S)-Iva-(Aib)$_4$-OMe (obtained by catalytic hydrogenation in MeOH of the corresponding Z derivative) and acetic anhydride in anhydrous MeCN at room temperature overnight. The sparingly soluble product was filtered off; yield 72%; m.p. 275–277°C (from MeOH/diethyl-ether); R_F(I) 0.20; R_F(II) 0.80; R_F(III) 0.05; $[\alpha]_D^{20}$ 1.6° (*c* 0.5, MeOH); $[\alpha]_{436}^{20}$ 3.7° (*c* 0.5, MeOH). IR (KBr): 3410, 3310, 3284, 1728, 1667, 1642, 1540 cm^{-1}. ^{1}H-NMR ($CDCl_3$, $1 \cdot 10^{-3}$ M): δ 7.48, 1s, 1H, NH; 7.36, s, 1H, NH; 7.24, s, 1H, NH; 6.25, s, 1H, NH; 5.71, s, 1H, NH; 3.69, s, 3H, OMe CH_3; 2.07, s, 3H, Ac CH_3; 1.80, m, 2H, Iva β-CH_2; 1.60-1.41, m, 27H, Aib and Iva β-CH_3; 0.90, m, 3H, Iva γ-CH_3.

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