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Conformation and Chiral Effects in α,β,α-Tripeptides

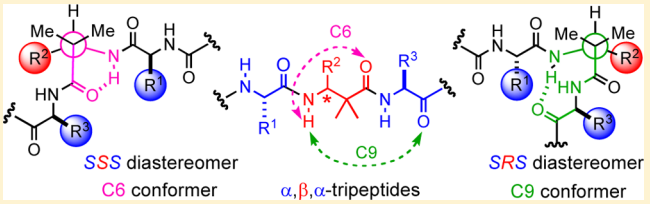
Carlos J. Saavedra,[†] Alicia Boto,*[,†] Rosendo Hernández,[†] José Ignacio Miranda,[‡] and Jesus M. Aizpurua*[,‡]

[†]Instituto de Productos Naturales y Agrobiología del CSIC, Avda. Astrofísico Francisco Sánchez 3, 38206 La Laguna, Tenerife, Spain

[‡]Departamento de Química Orgánica I, Jose Mari Korta R&D Center, Universidad del País Vasco UPV/EHU, Avda. Tolosa-72, 20018 San Sebastian, Spain

Supporting Information

ABSTRACT: Short α,β,α-tripeptides comprising a central chiral trisubstituted $\beta^{2,2,3}$*-amino acid residue form unusual γ-turns and δ-turns in $CDCl_3$ and DMSO-d_6 solutions but do not form β-turns. Thermal coefficients of backbone amide protons, 2D-NMR spectra, and molecular modeling revealed that these motifs were strongly dependent on the configuration (chiral effect) of the central β-amino acid residue within the triad. Accordingly, *SSS* tripeptides adopted an intraresidual γ-turn like (C6) arrangement in the central β-amino acid, whereas *SRS* diastereomers preferred an extended δ-turn (C9) conformation. A different *SRS*-stabilizing bias was observed in the crystal structures of the same compounds, which shared the extended δ-turn (C9) found in solution, but incorporated an additional extended β-turn (C11) to form an overlapped double turn motif.



INTRODUCTION

β-Amino acids exhibit a stricking ability to attain secondary structures far more efficiently than α-amino acids, and the term "foldamer"[1] has been coined for them and for some α,β-hybrids containing them.[2] As a result of their predictable folding patterns and their improved stability to proteolytic cleavage,[3] β-amino acid containing peptides are the peptidomimetics of choice in many biomedical applications.[4]

In the late 1990s, Gellman[5] and Seebach[6] established the general folding trends for β-peptides containing monosubstituted β^2- and β^3-amino acids or disubstituted $\beta^{2,3}$-amino acid units bearing proteinogenic side chains. Seebach also reported the conformational behavior of β-tripeptides constituted by geminally disubstituted $\beta^{2,2}$-amino acid residues. In the solid state, and depending on the nature of the $\beta^{2,2}$-substituents, such 3-mer peptides adopt either extended γ-turns (C8 hydrogen-bonded) or doubly extended δ-turns (C10), which are reminiscent of the canonical β-turns formed by α-amino acid tetrapeptides.[7] Introduction of a ^{D}Pro-$\beta^{3,3}$-amino acid α,β-dipeptide segment at the $(i-1)-(i+2)$ positions of a longer α-peptide stabilizes β-hairpins through extended β-turns (C11), either in the crystal or in methanol, as demonstrated by Balaram.[8] Far less attention has been drawn to trisubstituted $\beta^{2,2,3}$-amino acid containing hybrid α,β-peptides, despite the valuable protease inhibition properties shown by some of these compounds.[9]

Herein we report the first conformational study conducted in solution for model hybrid α,β,α-tripeptides **1**−**7** (Figure 1) containing a trisubstituted central $\beta^{2,2,3}$-amino acid residue with a single stereocenter at position β^3. The objective of this study was to establish the chiral effect exerted by the configuration of such stereocenter on the stabilization of several turned structures of the types C6−C11 (Figure 1) and to compare them to the C9 + C11 double hydrogen-bond pattern found in the solid state.[10]

According to crystal data (Figure 2), the *N*-(4-iodobenzoyl)-protected tripeptides **2** and **7** with a *SRS* configuration of the central triad feature identical double-turned motifs comprising an extended δ-turn (C9) and an extended β-turn (C11). The two overlapped turns share all the peptide chain atoms of the central $\beta^{2,2,3}$-amino acid residue and are further stabilized by intermolecular NH···O═C bonding. In the case of the C11 turn, a hydrogen bond forms between the 4-iodobenzoyl carbonyl oxygen and the Phe or Leu N_7H amides, whereas for the C9 turn the hydrogen bond is between the N_4H amide and the terminal ester carbonyl oxygen. The structures are further stabilized inside the crystal cell by additional intermolecular hydrogen bonds between the N_2H amide proton and the C═O oxygen of the β-amino acid residue of a contiguous molecule (not shown in Figure 2).

RESULTS AND DISCUSSION

The model tripeptides **1**−**7** were prepared as shown in Scheme 1 for compounds **3** and **4**. Thus, the α-dipeptide **8** underwent a one-pot radical decarboxylation−oxidation−alkylation process[10] to give the α,β-dipeptide **9** in good yield as a 2:1 diastereomer mixture. The saponification of the methyl ester, followed by coupling to H-Phe-OMe, afforded a separable mixture of α,β,α-tripeptides **3** (51%) and **4** (31%), which were used in the conformational studies. The other tripeptides **1**, **2**, and **5**−**7** have been previously reported.[10]

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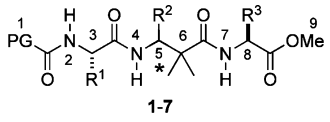
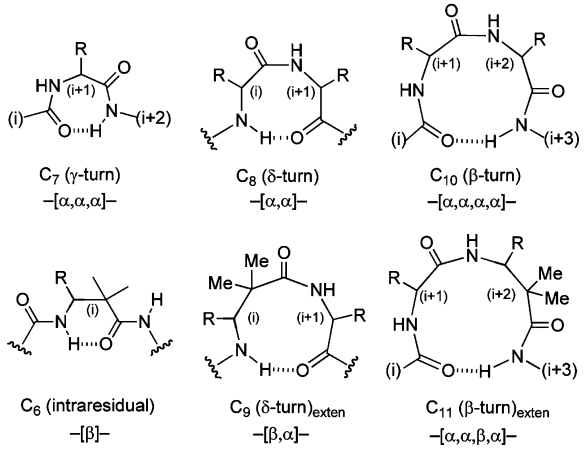


1-7

Peptide	PG	R^1(Aa)	R^2	R^3(Aa)	*	Triad
1	p-I-Bz	Me (Ala)	Me	Bn (Phe)	*S*	*SSS*
2	p-I-Bz	Me (Ala)	Me	Bn (Phe)	*R*	*SRS*
3	Bz	Me (Ala)	^{i}Bu	Bn (Phe)	*S*	*SSS*
4	Bz	Me (Ala)	^{i}Bu	Bn (Phe)	*R*	*SRS*
5	Cbz	^{i}Pr (Val)	Bn	^{i}Bu (Leu)	*S*	*SSS*
6	Cbz	^{i}Pr (Val)	Bn	^{i}Bu (Leu)	*R*	*SRS*
7	p-I-Bz	^{i}Pr (Val)	Bn	^{i}Bu (Leu)	*R*	*SRS*



C_7 (γ-turn) –[α,α,α]–

C_8 (δ-turn) –[α,α]–

C_{10} (β-turn) –[α,α,α,α]–

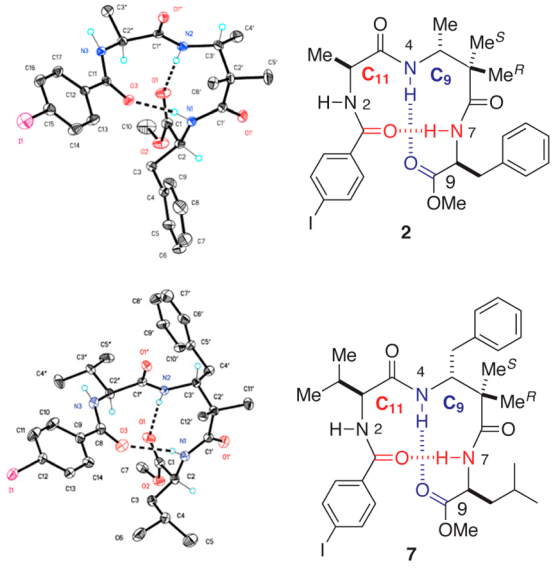
C_6 (intraresidual) –[β]–

C_9 (δ-turn)$_{exten}$ –[β,α]–

C_{11} (β-turn)$_{exten}$ –[α,α,β,α]–

Figure 1. Model *α,β,α*-tripeptides **1**–**7** selected for conformational analysis and turn motifs in short peptides, comprising *α*-amino acids (top) and combinations of *α*- and $\beta^{2,2,3}$*-amino acids (bottom). All structures are depicted as N→C sequences.

These model peptides **1**–**7** (Table 1) were designed to have different combinations of side chains (Me, *i*-Pr, *i*-Bu, Bn) with variable steric demand and hydrophobicity in order to study their interference with the intramolecular hydrogen bonding pattern in solution. Peptides **1**–**6** could be grouped into three pairs of

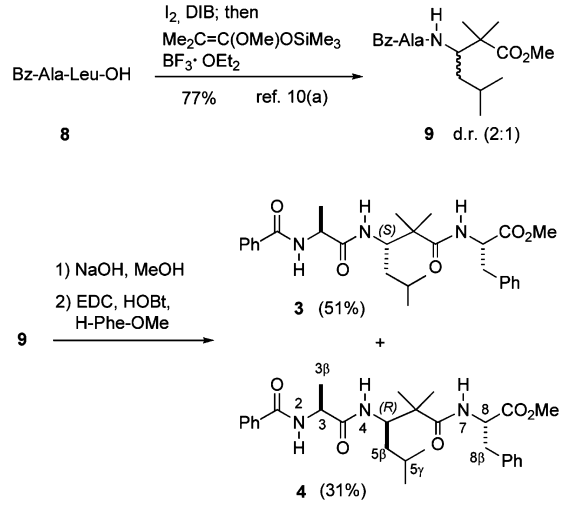


2

7

Figure 2. Crystal structures of *α,β,α*-tripeptides **2** and **7** containing a chiral trisubstituted central $\beta^{2,2,3}$*-amino acid residue. A (C9 + C11) hydrogen bonding pattern with two overlapped turns is formed in each case.

Scheme 1. Preparation of Model Tripeptides 3 and 4



diastereomers (**1**/**2**, **3**/**4**, and **5**/**6**) according to the (*S*) or (*R*) configuration of the central *β*-amino acid residue, but in all instances, the configuration of the flanking *α*-amino acids was *S* (*L*) to represent natural peptides. Finally, the tripeptides were protected at the N-termini by 4-iodobenzoyl, benzoyl, or Cbz groups and were capped at the C-termini as methyl esters. As mentioned above, the 4-iodobenzoylamido tripeptides of *SRS* configurations provided crystals (**2** and **7**) which were used to compare their C9 + C11 structures with the solution structures of **2** and **6** (the *p*-I-benzoyl analogue of compound **7**), respectively.

Although water was considered first as the most biologically meaningful medium for the study, finally less polar solvents were chosen for solubility reasons. Thus, each peptide was dissolved into DMSO-d_6 and $CDCl_3$ (5×10^{-3} M), and all protons were unambiguously assigned from COSY, TOCSY, HSQC, and HMBC spectra. Compounds **1**–**7** yielded sharp, well-resolved NMR spectra consisting of single sets of signals in both solvents. Experiments were then performed to determine the ^{1}H NMR temperature coefficients[11] for the exchangeable amide protons of the tripeptides in DMSO-d_6 solutions over a temperature range of 300–330 K at 5 K intervals (see Table 1). Vicinal coupling constants ^3J(HN–C*α*H) were also measured, and the corresponding *ϕ* dihedral angles were calculated using the Karplus equation.[12] Dihedral angles in the solid state for crystalline compounds **2** and **7** are also included in Table 1.

Relevant noncontiguous interproton distances for peptides **1**–**6** (Table 2; see also Supporting Information Table S1) were calculated following the ISPA (isolated spin-pair approximation) method[13] from the integration of key NOESY crosspeaks recorded in $CDCl_3$ solvent at 300 K (500 MHz) with mixing times of 400 ms. Interproton distances calculated in DMSO-d_6 solutions delivered values essentially identical to those obtained in $CDCl_3$ (see Table 2). Diagnostic interproton distances around the central *β*-amino acid residue are collected for peptides **1**, **2** and **5**, **6** in Figure 4.

Conformational Analysis. An inspection of the ^{1}H NMR spectra recorded from solutions of the tripeptides **1**–**7** in $CDCl_3$ or DMSO-d_6 soon revealed the absence of some key deshielded amide NH required to support the overlapped double turn conformations of **2** and **7** in the solid state (Figure 2), suggesting that the large extended *β*-turn (C11) of the crystals could break down when dissolved. Indeed, only the N_4H amide protons of the *β*-amino acid residues participated in the intramolecular

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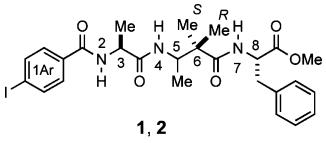
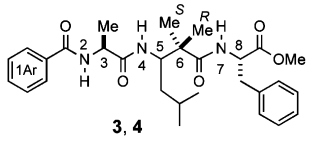
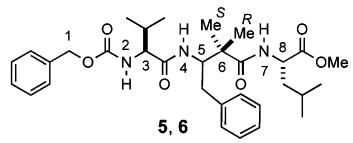
Table 1. Chemical shifts (δ), Amide NH Thermal Coefficients ($\Delta\delta/\Delta T$), and HN−CαH Dihedral Angles (ϕ) Measured for α,β,α-Tripeptides 1−7



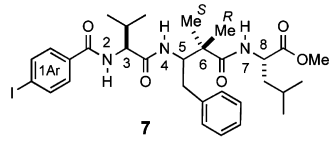
1, 2



3, 4



5, 6

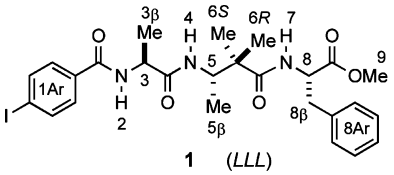


7

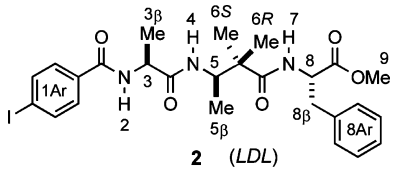
peptide	**1** (*SSS*)	**2** (*SRS*)	**3** (*SSS*)	**4** (*SRS*)	**5** (*SSS*)	**6** (*SRS*)	**7** (*SRS*)
$N_2H(\delta)^a$	7.42 (+1.11)	7.83 (+0.80)	7.08 (+1.34)	7.26 (+1.24)	5.00 (+2.00)	5.24 (+1.99)	6.71 (+1.25)
$N_4H(\delta)^a$	7.12 (+0.46)	7.67 (−0.14)	6.95 (+0.40)	7.19 (+0.17)	7.17 (+0.26)	7.56 (+0.00)	7.72 (+0.11)
$N_7H(\delta)^a$	6.15 (+1.66)	7.22 (+0.55)	6.17 (+1.60)	7.41 (+0.30)	6.17 (+1.84)	7.16 (+0.65)	7.48 (+0.92)
$N_2H(\Delta\delta/\Delta T)^b$	−4.5	−4.9	−4.0	−4.8	−5.9	−6.5	−5.2
$N_4H(\Delta\delta/\Delta T)^b$	−3.7	−3.8	−4.0	−4.6	−3.7	−4.9	−4.7
$N_7H(\Delta\delta/\Delta T)^b$	−5.9	−5.6	−6.4	−5.4	−6.9	−5.4	−6.0
$^3J(\phi\ HN_2{-}C_3H)^c$	6.8 (−140)	6.3 (−136)[−149]^d	5.9 (−133)	7.4 (−142)	8.1 (−152)	7.9 (+147)	7.9 (+147)[−158]^d
$^3J(\phi\ HN_4{-}C_5H)^c$	9.2 (+164)	10.2 (+180)[+137]^d	10.5 (+180)	9.8 (+180)	9.8 (+180)	10.2 (+174)	10.0 (+170)[+166]^d
$^3J(\phi\ HN_7{-}C_8H)^c$	7.5 (−146)	7.9 (−150)[−167]^d	7.8 (−151)	7.7 (−148)	7.5 (+146)	7.8 (+147)	7.9 (+149)[−148]^d

^{a}Chemical shifts (ppm) measured in $CDCl_3$. Values in parentheses represent variations of the chemical shift when the $CDCl_3$ solvent was changed to DMSO-d_6. ^{b}Thermal coefficients in ppb/K measured in DMSO-d_6. ^{c}Coupling constants (J) in Hz. The ϕ dihedral angles, in parentheses, were calculated from the Karplus equation. ^{d}Dihedral angles, in brackets, from X-ray data, ref 10a.

Table 2. Key Interproton Distances (Å) Calculated from NOESY^a Experiments for α,β,α-Tripeptides 1 and 2 in $CDCl_3$ and DMSO-d_6 Solutions



1 (*LLL*)



2 (*LDL*)

NOE	Ha-Hb^b	**1** ($CDCl_3$)	**1** (DMSO-d_6)	**2** ($CDCl_3$)	**2** (DMSO-d_6)	**2** (X-ray)^c
1	$Ar_1^{ortho}{-}N_2H$	2.16^{ref}	2.16^{ref}	2.16^{ref}	2.16^{ref}	[2.2]
2	$N_2H{-}C_3H$	2.9	3.0	3.8	4.0	[2.7]
3	$N_2H{-}C_{3\beta}Me$	2.4	2.4	2.4	2.5	[2.6]
4	$C_{3\beta}Me{-}N_4H$	4.1	4.1		3.8	[3.1]
5	$N_4H{-}C_5H$	3.3	3.1	3.9	4.3	[2.7]
6	$N_4H{-}C_{5\beta}Me$	2.6	3.0		3.1	[2.6]
7	$N_4H{-}C_6Me^R$	4.2		2.7	2.8	[3.0]
8	$N_4H{-}C_6Me^S$	2.7	2.9	4.5	4.1	[4.3]
9	$N_4H{-}N_7H$			2.7	2.9	[2.3]
10	$C_5H{-}C_6Me^R$	2.5	2.7	2.6	2.2	[2.5]
11	$C_5H{-}C_6Me^S$	2.5	3.1	2.6	2.8	[2.5]
12	$N_7H{-}C_6Me^R$	2.5	2.5	2.2	2.8	[2.6]
13	$N_7H{-}C_6Me^S$	3.0	2.7	2.9	2.8	[4.0]
14	$N_7H{-}C_8H$	2.6		4.5	3.4	[2.5]
15	$N_7H{-}C_{8\beta}H$	3.7		2.6	4.1	[2.5]
16	$Ar_8H{-}C_9(OMe)$	3.6			4.3	

^{a}Experiments (500 MHz) carried out at 300 K, mixing time 400 ms. ^{b}Reference distance. ^{c}Crystal interproton distances from X-ray analysis of **2** (C9 + C11 conformer).

hydrogen bond network of the peptides in solution, while the N_7H amide protons were exposed to the solvent. This was evident from the analysis of thermal coefficients in DMSO-d_6[11] (Table 1, rows 4−6) showing absolute values in the range −3.7 to −4.9 ppb/K for N_4H protons, whereas amides N_2H and N_7H gave larger thermal coefficients (up to −6.9 ppb/K). Solvent change from nonacceptor $CDCl_3$ to acceptor DMSO-d_6 resulted in very small chemical shift for β-amino acid N_4H amides in all peptides (0.0−0.4 ppm), but in a significantly larger downfield shift (0.4−1.9 ppm) for α-amino acid amides N_2H and N_7H (Table 1, rows 1−3). Seeking for more insight into the origin of the chiral effect differentiating the *SSS* conformers (**1**, **3** and **5**) and *SRS* conformers (**2**, **4**, **6**, and **7**), we noticed that the chemical shift of amide N_7H protons experienced different downfield displacement upon solvent changing from $CDCl_3$ to DMSO-d_6, depending on the β-amino acid β-carbon configuration. Thus, N_7H amide peak shifted downfield by 1.7−1.8 ppm in peptides **1**, **3** and **5** (*SSS* configuration), whereas peptides **2**, **4**, **6**, and **7** (*SRS* configuration) shifted only 0.3−0.6 ppm. This lower exposition to coordinating solvent suggests a conformation with the N_7H group oriented toward the inner side of the peptide turn backbone in *SRS* diastereomers.

All of the ϕ dihedral angles measured in solution for the vicinal (HN−CαH) atoms in the three amino acids of peptides **1**−**7**

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showed large values, in the range 133−180°, and were compatible with quasi-staggered dispositions of the HN−CH bonds (Table 1, rows 7−9). They were also very close to the dihedral angles of the crystalline peptides **2** and **7** (Table 1, values in brackets) suggesting that, after the solution of tripeptides **1**−**7** in $CDCl_3$ or DMSO-d_6, the relative spatial arrangement of the α-amino acid residues around the β-amino acid should resemble an open angular shape in *SSS* diastereomers and a closed turned shape in *SRS* diastereomers (Figure 3). These diastereomeric

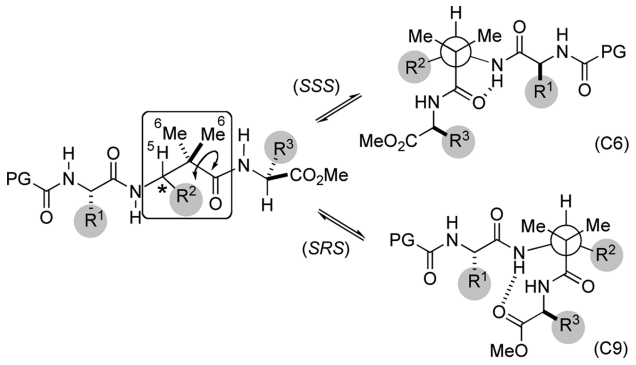

Figure 3. Main folding pattern (chiral effect) for peptides **1**−**6** in solution.

conformers, arising from rotations of the (Me_2C−CO) bond in the central β-amino acid residue, would be spatially directed by the arrangement of the R^2 substituent attached to the β^3 chiral stereocenter *C_5H and further stabilized with the formation of two different intramolecular hydrogen bonds (C6) and (C9).

An examination of the noncontiguous interproton distances collected in Figure 4 (see also Table 2 and Supporting Information Table S1) allowed the identification of some diagnostic NOESY crosspeaks around the central β-amino acid residue to discriminate the prevalent conformations of *SSS* and *SRS* diastereomers in tripeptides **1**, **2** and **5**, **6**. Unfortunately, some key peaks of tripeptides **3** and **4** (e.g., the β-amino acid geminal methyl groups) were overlapped in the ^{1}H NMR spectra, and these compounds were discarded from NOESY-based interproton distance analysis.

In the β-amino acid residue of peptides **1**, **2** and **5**, **6**, distances between the C_5H proton and the geminal diastereotopic methyl groups ($Me_6^{pro\text{-}R}$/$Me_6^{pro\text{-}S}$) were very similar to one another in all instances, either in solution or in the solid state. This confirmed the antiperiplanar disposition of the C_5−H proton and the β-amino acid's carbonyl group as the most stable and largely preferred conformation of the central residue in these tripeptides. Likewise, the distance between the N_7H amide proton and the geminal methyl groups at position C_6 showed a similar pattern in all instances, with the N_7H proton significantly closer to the $Me_6^{pro\text{-}R}$ group than to the $Me_6^{pro\text{-}S}$ group. In the crystal, peptides **2** and **7** had a large N_7H − $Me_6^{pro\text{-}S}$ distance of ~4.0 Å, which permitted the involvement of the N_7H amide proton in an extended β-turn (C11). Upon solution in $CDCl_3$ such hydrogen bond vanished in *SRS* isomers, the N_7H amide proton approached the $Me_6^{pro\text{-}R}$ group and only the more stable (C9) extended δ-turn remained (Figure 4). Conversely, the conformation of *SSS* tripeptides **1** and **5** was stabilized by an unusual intrarresidual (C6) H-bond.

Assuming (C6) and (C9) preferred conformations for α,β,α-tripeptides **1**−**6** in solution, the N_4H/N_7H and N_4H/$Me_6^{pro\text{-}R}$ pairs of protons were anticipated to be separated from each other

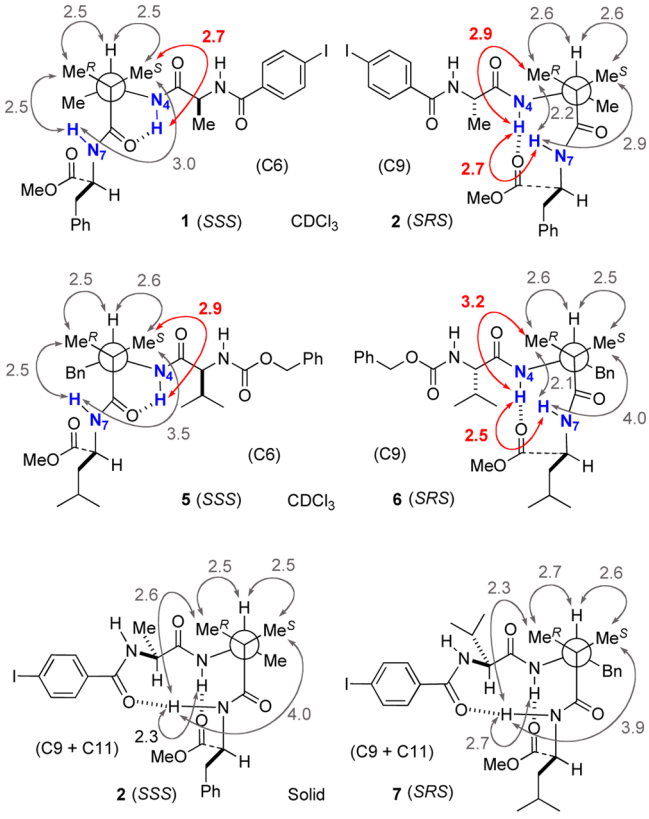

Figure 4. NMR−NOESY interproton distances (in Å) and main conformation structures for peptides **1**, **2** and **5**, **6** in $CDCl_3$ solution and in the solid state (for **2** and **7**). Diagnostic distances are highlighted in red.

in *SSS* tripeptides **1** and **5** but much closer in *SRS* diastereomers **2** and **6**, thus being suitable for diagnostic NOE analysis. As shown in Figure 5 for the NOE signals of peptides **1** and **2**, the crosspeaks between N_4H/N_7H amide protons (NOE **f**) were actually found in the NOESY spectrum of **2**, integrating for a 2.5 Å distance, whereas these crosspeaks were absent in the NOESY spectra of the *SSS* tripeptide **1** (for analogous spectra of **5** and **6**, see the Supporting Information, Figure S13). Consistent with the proposed conformers, strong NOE crosspeaks for N_4H amide protons with $Me_6^{pro\text{-}R}$ group were also found only in the *SRS* tripeptides **2** and **6**, but not in the *SSS* diastereomer counterparts **1** and **5**. In the later tripeptides, the N_4H amide protons gave strong NOE interactions only with the diastereotopic $Me_6^{pro\text{-}S}$ protons (see NOE **d** in Figure 5).

No significant additional long-range inter-residual NOE crosspeaks were found in the NOESY spectra of peptides **1**−**6**. Furthermore, the Molecular Mechanics minimization of **1**−**6** including the NMR interproton distance restrictions and ϕ dihedral angles measured in solution, yielded essentially single-conformer structures in each case. These observations collectively suggested that the chiral conformational bias observed for tripeptides **1**−**6** in solution is roughly independent of the size and nature of the R^1, R^2 and R^3 substituents and, most important, that incorporation of α,β,α-peptide segments with a $\beta^{2,2,3}$*-amino acid in longer peptides could be envisaged to prepare novel peptidomimetics with predictable shapes in solution (Figure 6).[14]

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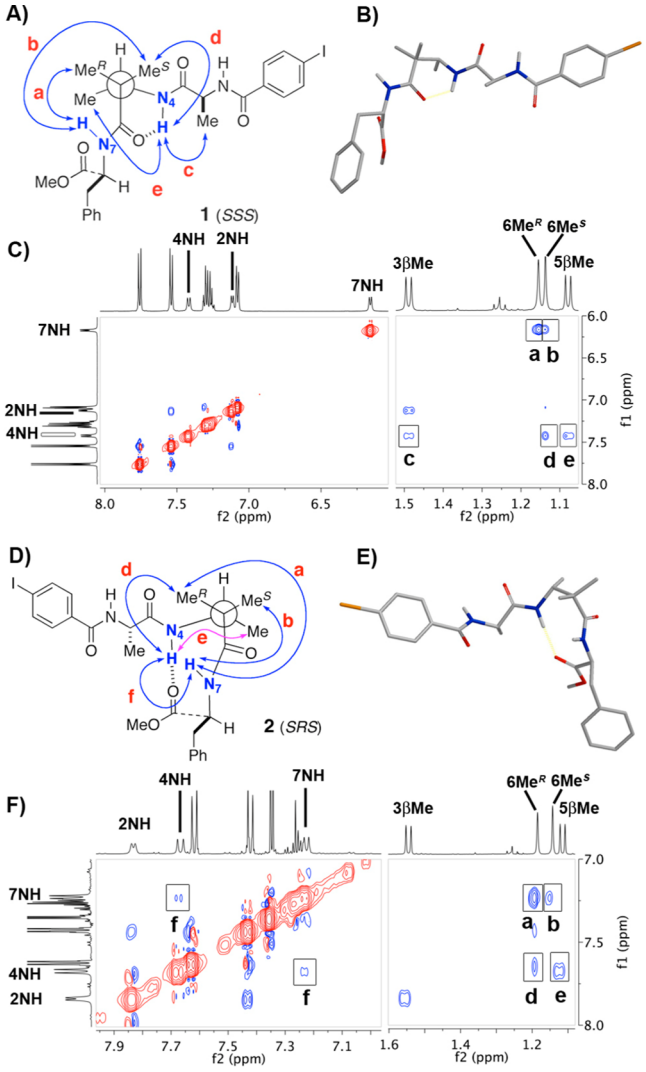

Figure 5. Diagnostic NOE interactions for peptides **1** and **2**: Spatially close protons in **1** (A) and **2** (D). Molecular Mechanics (SYBIL force field) minimum energy conformers of **1** (B) and **2** (E) restrained with NMR interproton distances and ϕ dihedral angles (only amide protons are shown for clarity). Expansions of key NOESY crosspeaks for **1** (C) and **2** (F). For position numbering, see structures in Table 2.

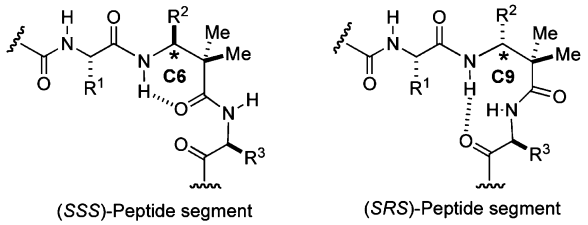

Figure 6. General intramolecular hydrogen-bond patterns populated in solution for α,β-hybrid peptides containing a central $\beta^{2,2,3}$* chiral residue.

CONCLUSIONS

A uniform chiral effect was found to dictate the conformational behavior of α,β,α-tripeptides containing a central $\beta^{2,2,3}$*-amino acid residue with a single stereocenter at β^3 position when such peptides were dissolved in $CDCl_3$ and DMSO-d_6 solvents. Tripeptides with homochiral *SSS* relative configuration populate in solution a conformation characterized by an unusual (C6) turn H-bonded between the N−H and C═O groups of the central β-amino acid residue. Conversely, tripeptides with heterochiral *SRS* relative configuration stabilize an extended δ-turn with a (C9) hydrogen bond between the N−H of the β-amino acid residue and the C═O of the next α-amino acid. These results, based on amide NH thermal coefficient measurements, diagnostic NOESY crosspeak analysis and computational modeling, also evidenced significant differences with the solid state conformations previously reported for similar heterochiral *SRS* tripeptides, which were characterized by the additional stabilization of the extended δ-turn (C9) with an extended β-turn (C11) spanning from the C═O of the protecting group and the N−H of the C-terminal α-amino acid.

EXPERIMENTAL SECTION

General Experimental Methods. Commercially available reagents and solvents were analytical grade or were purified by standard procedures prior to use. All reactions involving air- or moisture-sensitive materials were carried out under nitrogen atmosphere. The spray reagents for TLC analysis were 0.25% ninhydrin in ethanol and/or Fleet's reagent [$Ce(SO_4)_2$ (0.5 g) and ammonium phosphomolybdate hydrate (2.5 g) in H_2SO_4 (5 mL) and water (65 mL)]. Once sprayed, the TLC was heated until development of color. Merck silica gel 60 PF_{254} and 60 (0.063−0.2 mm) were used for rotatory chromatography and column chromatography, respectively. Melting points were determined with a hot-stage apparatus and are uncorrected. Optical rotations were measured at the sodium line at ambient temperature (26 °C). NMR spectra were determined at 500 MHz for ^{1}H and 125.7 MHz for ^{13}C, and Mass spectra (EI) were determined at 70 eV using an ion trap mass analyzer. ^{1}H NMR references: $CDCl_3$ (δ 7.26), DMSO (δ 2.49). ^{13}C NMR references: $CDCl_3$ (δ 77.0), DMSO (δ 39.5).

Preparation of Compounds 1−7. The preparation and spectroscopic data of compounds **1**, **2**, and **5**−**7** was reported previously.[10a] The syntheses of compounds **3** and **4**, and their precursor **9**, are reported below.

*N-Benzoyl-L-alanyl-α,α-dimethyl-(S)-β-homoleucine Methyl Ester (**9**).* To a solution of Bz-Ala-Leu-OH (**8**) (61 mg, 0.2 mmol) in dry dichloromethane (6 mL) were added iodine (15 mg, 0.06 mmol) and diacetoxyiodobenzene (DIB) (97 mg, 0.3 mmol). The reaction mixture was stirred at 26 °C for 4 h under irradiation with visible light. Then the solution was cooled to 0 °C, and methyl (trimethylsilyl)dimethylketene acetal (203 μL, 174 mg, 1.0 mmol) was injected, followed by dropwise addition of $BF_3{\cdot}OEt_2$ (51 μL, 57 mg, 0.4 mmol). The mixture was allowed to reach room temperature and stirred for 3 h; it was then poured into 10% aqueous $Na_2S_2O_3$/saturated aqueous $NaHCO_3$ (1:1, 10 mL) and extracted with CH_2Cl_2. The organic layer was dried on sodium sulfate, filtered, and evaporated under vacuum. The residue was purified by rotatory chromatography 85:15 (hexanes/ethyl acetate mixtures) to give the product **8** (56 mg, 77%) as a 2:1 diastereomer mixture: amorphous solid; IR ($CHCl_3$) ν_{max} 3424, 3323, 1721, 1676, 1652, 1511, 1484 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H major diastereomer: 0.75 (3H, d, J = 6.6 Hz, 5γ-Me_a), 0.84 (3H, d, J = 6.3 Hz, 5γ-Me_b), 1.19 (3H, s, 6-Me_a), 1.19−1.35 (2H, m, 5β-H_2), 1.20 (3H, s, 6-Me_b), 1.49 (1H, m, 5γ-H), 1.53 (3H, d, J = 6.9 Hz, 3-Me), 3.66 (3H, s, OMe), 4.15 (1H, m, 5-H), 4.78 (1H, m, 3-H), 6.83 (1H, br d, J = 9.8 Hz, NH_{Leu}), 7.21 (1H, br d, J = 7.3 Hz, NH_{Ala}), 7.40 (2H, dd, J = 7.3, 7.9 Hz, Ar), 7.48 (1H, dd, J = 7.6, 7.8 Hz, Ar), 7.78 (2H, d, J = 8.5 Hz, Ar); minor isomer: 0.88 (3H, d, J = 6.6 Hz, 5γ-Me_a), 0.90 (3H, d, J = 6.3 Hz, 5γ-Me_b), 1.11 (3H, s, 6-Me_a), 1.15 (3H, s, 6-Me_b), 1.11−1.20 (2H, m, 5β-H_2), 1.51 (3H, d, J = 6.9 Hz, 3-Me), 1.57 (1H, m, 5γ-H), 3.63 (3H, s, OMe), 4.15 (1H, m, 5-H), 4.78 (1H, m, 3-H), 6.81 (1H, br d, J = 8.5 Hz, NH_{Leu}), 7.23 (1H, br d, J = 9.1 Hz, NH_{Ala}), 7.39 (2H, dd, J = 7.9, 8.0 Hz, Ar), 7.47 (1H, dd, J = 7.6, 7.8 Hz, Ar), 7.79 (2H, d, J = 8.7 Hz, Ar); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C major diastereomer: 19.2 (CH_3), 21.32 (CH_3), 22.1 (CH_3), 23.1 (CH_3), 23.7 (CH_3), 25.0 (CH), 40.1 (CH_2), 46.7 (C), 49.5 (CH), 51.8 (CH_3), 53.3 (CH), 127.0 (2 × CH), 128.5 (2 × CH), 131.7 (CH), 133.9 (C), 167.1 (C), 172.2 (C), 177.0 (C); minor diastereomer: 18.8 (CH_3), 21.27 (CH_3), 21.9 (CH_3), 23.3 (CH_3), 23.8

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(CH_3), 25.2 (CH), 40.3 (CH_2), 46.5 (C), 49.5 (CH), 51.8 (CH_3), 53.3 (CH), 127.0 (2 × CH), 128.5 (2 × CH), 131.7 (CH), 133.8 (C), 167.1 (C), 172.4 (C), 177.1 (C); MS (EI) *m*/*z* (rel intensity) 363 (M^+ + H, 2), 176 ([PhCONHCH(Me)CO]$^+$, 25), 149 ([PhCONHCH(Me) + H]$^+$, 53), 148 ([PhCONHCH(Me)]$^+$, 62), 105 ([PhCO]$^+$, 100), 86 ([$NH_2CHCH_2CHMe_2$], 58); HRMS (EI) calcd for $C_{20}H_{31}N_2O_4$ 363.2284, found 363.2270; calcd for $C_{10}H_{10}NO_2$ 176.0712, found 176.0707; calcd for $C_9H_{11}NO$ 149.0841, found 149.0841; calcd for $C_9H_{10}NO$ 148.0762, found 148.0758; calcd for C_7H_5O 105.0340, found 105.0337; calcd for $C_5H_{12}N$, 86.0970, found 86.0967. Anal. Calcd for $C_{20}H_{30}N_2O_4$: C, 66.27; H, 8.34; N, 7.73. Found: C, 66.26; H, 8.35; N, 7.37.

*N-Benzoyl-L-alanyl-[α,α-dimethyl-(S)-β-homoleucyl]-L-phenylalanine Methyl Eester (**3**) and N-Benzoyl-L-alanyl-[α,α-dimethyl-(R)-β-homoleucyl]-L-phenylalanine Methyl Ester (**4**).* To a solution of the dipeptide mixture **8** (120 mg, 0.33 mmol) in methanol (7 mL) at 0 °C was slowly added 2 N aqueous NaOH (3 mL). The reaction mixture was allowed to reach 26 °C and stirred for 64 h, and then it was cooled to 0 °C, diluted with water, poured into 5% HCl, and extracted with EtOAc. The organic layer was dried and evaporated, and the residue was dissolved in dry CH_2Cl_2 (4 mL) and treated with L-phenylalanine methyl ester hydrochloride (70 mg, 0.33 mmol). The solution was cooled to 0 °C, and Et_3N (45 μL, 33 mg, 0.33 mmol), EDC (69 mg, 0.36 mmol), and HOBt (49 mg, 0.36 mmol) were added. The reaction mixture was stirred at 0 °C for 2 h, and then it was allowed to reach room temperature, stirred for 18 h, and finally poured into a saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. After usual drying and solvent removal, the residue was purified by rotatory chromatography (hexanes/EtOAc, 65:35), affording compounds **3** (86 mg, 51%) and **4** (52 mg, 31%).

*Compound (**3**):* amorphous solid; $[\alpha]_D$ +26 (0.43, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3439, 3420, 1741, 1652, 1505 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 0.81 (3H, d, *J* = 6.6 Hz, 5γ-Me_a), 0.85 (3H, d, *J* = 6.6 Hz, 5γ-Me_b), 1.13 (3H, s, 6-Me_a), 1.15 (3H, s, 6-Me_b), 1.15−1.27 (2H, m, 5β-H_2), 1.48 (1H, m, 5γ-H), 1.50 (3H, d, *J* = 6.9 Hz, 3-Me), 3.07 (1H, dd, *J* = 6.3, 13.9 Hz, 8β-H_a), 3.15 (1H, dd, *J* = 5.7, 13.9 Hz, 8β-H_b), 3.75 (3H, s, OMe), 3.90 (1H, ddd, *J* = 2.8, 11.1, 11.3 Hz, 5-H), 4.63 (1H, dddd, *J* = 6.9, 6.9, 6.9, 6.9 Hz, 3-H), 4.78 (1H, ddd, *J* = 6.0, 6.3, 7.6 Hz, 8-H), 6.11 (1H, br d, *J* = 7.6 Hz, N_7H [NH_{Phe}]), 6.94 (1H, br d, *J* = 9.8 Hz, N_4H [NH_{Leu}]), 7.06 (1H, br d, *J* = 6.6 Hz, N_2H [NH_{Ala}]), 7.08 (2H, d, *J* = 6.8 Hz, Ar), 7.25−7.31 (3H, m, Ar), 7.41 (2H, dd, *J* = 6.6, 7.2 Hz, Ar), 7.49 (1H, dd, *J* = 7.3, 7.6 Hz, Ar), 7.80 (2H, d, *J* = 7.1 Hz, Ar); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ_C 19.2 (CH_3), 21.4 (CH_3), 23.2 (CH_3), 23.8 (CH_3), 24.4 (CH_3), 25.0 (CH), 37.5 (CH_2), 40.1 (CH_2), 45.6 (C), 49.5 (CH), 52.4 (CH_3), 52.8 (CH), 54.9 (CH), 127.1 (2 × CH), 127.3 (CH), 128.5 (2 × CH), 128.7 (2 × CH), 129.2 (2 × CH), 131.5 (CH), 134.2 (C), 135.8 (C), 166.9 (C), 171.9 (2 × C), 176.4 (C); MS (EI) *m*/*z* (rel intensity) 509 (M^+, 1), 361 (M^+ − PhCONHCHMe, 15), 249 ([Me_2CCONHCH(CH_2Ph)CO_2Me + H]$^+$, 39), 148 ([PhCONHCH-(Me)]$^+$, 32), 105 ([PhCO]$^+$, 100), 86 ([$NH_2CHCH_2CHMe_2$], 95); HRMS (EI) calcd for $C_{29}H_{39}N_3O_5$ 509.2890, found 509.2888; calcd for $C_{20}H_{29}N_2O_4$ 361.2127, found 361.2120; calcd for $C_{14}H_{19}NO_3$ 249.1365, found 249.1356; calcd for $C_9H_{10}NO$ 148.0762, found 148.0764; calcd for C_7H_5O 105.0340, found 105.0342; calcd for $C_5H_{12}N$ 86.0970, found 86.0967. Anal. Calcd for $C_{29}H_{39}N_3O_5$: C, 68.34; H, 7.71; N, 8.25. Found: C, 68.65; H, 7.86; N, 8.22.

*Compound (**4**):* amorphous solid; $[\alpha]_D$ +38 (0.17, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3442, 3362, 1731, 1652 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 0.88 (3H, d, *J* = 6.6 Hz, 5γ-Me_a), 0.90 (3H, d, *J* = 6.6 Hz, 5γ-Me_b), 1.11 (3H, s, 6-Me_a), 1.12 (3H, s, 6-Me_b), 1.23 (1H, ddd, *J* = 3.8, 12.0, 15.4 Hz, 5β-H_a), 1.34 (1H, ddd, *J* = 2.8, 10.1, 14 Hz, 5β-H_b), 1.52 (3H, d, *J* = 6.9 Hz, 3-Me), 1.56 (1H, m, 5γ-H), 3.23 (2H, d, *J* = 6.8 Hz, 8β-H_2), 3.78 (3H, s, OMe), 3.89 (1H, ddd, *J* = 2.8, 11.0, 11.7 Hz, 5-H), 4.46 (1H, dddd, *J* = 6.9, 6.9, 6.9, 6.9 Hz, 3-H), 4.78 (1H, ddd, *J* = 6.3, 6.6, 8.0 Hz, 8-H), 7.15 (1H, d, *J* = 8.2 Hz, N_7H [NH_{Phe}]), 7.24 (1H, br b, N_2H [NH_{Ala}]), 7.24−7.37 (8H, m, Ar + N_4H [NH_{Leu}]), 7.47 (1H, dd, *J* = 7.3, 7.5 Hz, Ar), 7.76 (2H, d, *J* = 6.9 Hz, Ar); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ_C 17.6 (CH_3), 21.3 (CH_3), 22.6 (CH_3), 23.9 (2 × CH_3), 25.3 (CH), 36.6 (CH_2), 38.8 (CH_2), 46.5 (C), 50.8 (CH), 52.7 (CH_3), 54.3 (CH), 54.5 (CH), 126.9 (CH), 127.1 (2 × CH), 128.5 (4 × CH), 129.2 (2 × CH), 131.6 (CH), 133.6 (C), 137.0 (C), 167.6 (C), 172.8 (C), 174.5 (C), 175.6 (C); MS (EI) *m*/*z* (rel intensity) 509 (M^+, 1), 361 (M^+ − PhCONHCHMe, 20), 249 ([Me_2CCONHCH(CH_2Ph)CO_2Me + H]$^+$, 60), 148 ([PhCONHCH(Me)]$^+$, 36), 105 ([PhCO]$^+$, 100), 86 ([$NH_2CHCH_2CHMe_2$], 70); HRMS (EI) calcd for $C_{29}H_{39}N_3O_5$ 509.2890, found 509.2882; calcd for $C_{20}H_{29}N_2O_4$ 361.2127, found 361.2126; calcd for $C_{14}H_{19}NO_3$ 249.1365, found 249.1358; calcd for $C_9H_{10}NO$ 148.0762, found 148.0767; calcd for C_7H_5O 105.0340, found 105.0336; calcd for $C_5H_{12}N$ 86.0970, found 86.0970. Anal. Calcd for $C_{29}H_{39}N_3O_5$: C, 68.34; H, 7.71; N, 8.25. Found: C, 68.64; H, 7.83; N, 8.20.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR, ^{13}C NMR, and NOESY spectroscopic data of compounds **1**−**6** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: (J.M.A.) jesusmaria.aizpurua@ehu.es, (A.B.) alicia@ipna.csic.es.

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(14) NMR structural analyses conducted on α,β,α-tripeptides of the type Cbz-Pro-(HN-CH(Bn)CMe_2CO)-Leu-OMe were also attempted, but the additional conformation constraint imposed by the N,N-disubstituted proline residue resulted in multiple sets of signal in the ^{1}H NMR spectra and coalescence temperatures above 50 °C which prevented the proper determination of the NH amide thermal coefficients.