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Azetidine-Derived Amino Acids versus Proline Derivatives. Alternative Trends in Reverse Turn Induction

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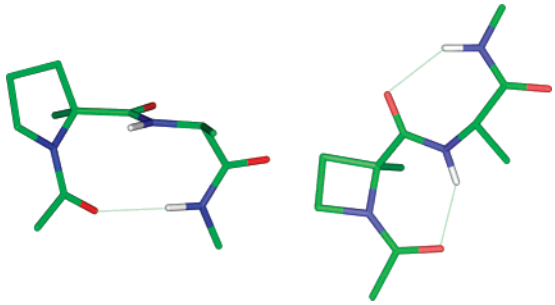

The influence of 2-alkyl-2-carboxyazetidines (Aze) on the 3D structure of model tetrapeptides R^2CO-2-R^1Aze-L-Ala-NHMe has been analyzed by molecular modeling, ^{1}H NMR, and FT-IR studies. The conformational constraints introduced by the four-membered ring resulted in an effective way to stabilize γ-turn-like conformations in these short peptides. The conformational preferences of these Aze-containing peptides have been compared to those of the corresponding peptide analogues containing Pro or α-MePro in the place of 2-alkyl-Aze residue. In the model studied, both Pro and Aze derivatives are able to induce reverse turns, but the nature of the turn is different as a function of the ring size. While the five-membered ring of Pro tends to induce β-turns, as previously suggested by different authors, the four-membered ring of Aze residues forces the peptide to preferentially adopt γ-turn conformations. In both cases, the presence of an alkyl group at the α-position of Pro or the azetidine-2-carboxylate ring enhances significantly the turn-inducing ability. These results might open the opportunity of using 2-alkyl-Aze residues as versatile tools in defining the role of γ-turn structures within the bioactive conformation of selected peptides, and represent an alternative to Pro derivatives as turn inducers.

Introduction

Protein−protein and peptide−protein interactions play key roles in most biological processes, and therefore represent valuable targets for drug discovery. As these interactions require a high degree of surface complementarity and precision in binding site docking, a valuable approach in the search of new chemical entities able to interfere with these interactions is the use of small compounds able to mimic or to induce precise aspects of specific peptide secondary structures.[1−3] Among the secondary structure elements, reverse turns have been shown to be relevant in biomolecular recognition events, mainly due to their frequent localization in the exposed surface of peptides and proteins.[4−7] Thus, turn-like hot-spots have been recognized within several protein−protein interaction surfaces,[6,8] and it has recently been described that over one hundred G protein coupled receptors bind to their respective peptide/protein ligands through turn motifs.[9] The most prevalent reverse turn in peptides and proteins is the β-turn, which is defined as any tetrapeptide

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sequence in a nonhelical region with a distance from the α-carbons of the first and fourth residues ($\alpha C^{i}-\alpha C^{i+3}$) lower than 7 Å.[6,10−12] The γ-turn is the second most characterized and commonly found turn, after the β-turn. γ-Turns, consisting of three consecutive amino acids structured in a seven-membered pseudocycle, have also been implicated in several biological events.[6,10,11,13] Structurally, β- and γ-turns are frequently stabilized by an intramolecular H-bond between the backbone CO of the first residue (i) and the backbone NH of the fourth ($i + 3$) and the third ($i + 2$) residue, respectively.[6,11,14]

It is known that proline serves as a turn inducer in natural peptides and proteins, with a high frequency of occurrence in the ($i + 1$) position of β-turn motifs.[15,16] Moreover, it has been shown that the α-alkylation of Pro, as in α-MePro, provides further stabilization of β-turn conformations in linear peptides.[17] Conformational studies carried out on its lower homologue, the natural amino acid azetidine-2-carboxylic acid (Aze),[15,18−22] have pointed out the propensity for β-bend formation of the Aze-containing peptides, but with higher flexibility compared to Pro analogues. We have recently performed a conformational analysis of 2-alkyl-2-carboxy azetidines, when incorporated at the $i + 1$ position of simplified model tetrapeptides, and showed that these non-proteinogenic amino acids possess an unexpected ability to induce γ-turn conformations.[23] Thus, contrary to Pro, α-MePro, and Aze that tend to fix β-turns, the α-alkylation of azetidine-containing amino acids seems to change the conformational preferences, favoring γ-turns. However, the encountered discrepancies might be due to the different peptide models used in the reported studies, or to the different parameters measured to determine the conformation preferences in each case.

We are now interested in further exploring the effects of the alkylation at position 2 of the azetidine ring on peptide conformation, and in comparing the results with those obtained for the non-alkylated amino acid (Aze) and their higher homologues Pro and α-MePro. As it is known that the amino acids appended to these residues might influence their secondary structure propensities, a unique peptide model, and the same conformational studies have to be used for reaching consistent conclusions. Thus, a series of dipeptides RCO-Xaa-Ala-NHMe (Xaa = Pro, α-MePro, Aze, 2-MeAze, 2-BnAze) have now been synthesized and studied for their conformational preferences by means of molecular modeling, IR-FT, and NMR. These dipeptides could be considered as simplified tetrapeptide models, in which the *N*-terminal amino acid has been substituted by different acyl groups (R = OBn, Me, ^{t}Bu) and the *C*-terminal residue by an *N*-methyl moiety. The present results fully agree with our preliminary conclusions and provide strong support for Aze amino acid derivatives as complementary tools to Pro analogues for inducing reverse turns.

Results and Discussion

Synthesis. Noncommercially available Z-α-MePro-OH (**1b**) was obtained from H-α-L-MePro-OH by acylation with benzyloxycarbonyl chloride. Boc-Aze-OH (**2a**) was from commercial sources, and starting azetidine derivatives Z-2-MeAze-OH ((*R*,*S*)-**3b**), Piv-2-MeAze-OH ((*R*,*S*)-**4b**), and Z-2-BnAze-OH ((*R*,*S*)-**3c**) were prepared following a synthetic procedure previously developed in our laboratory (see the Supporting Information for details).[24−26]

Two main approaches, following standard solution peptide coupling procedures, were initially investigated for the synthesis of model peptide derivatives (Scheme 1). Approach A consisted of the formation of the dipeptide methyl ester, transformation into the corresponding *N*-methyl amide, removal of the Z group, and incorporation of the acetyl or pivaloyl moiety. Using the reverse sequence of reactions, in Approach B the *N*-Boc-protected dipeptide **6a** was treated with HCl, to remove the Boc group, followed by the incorporation of the selected acyl moieties (Z, Ac, Piv), and final formation of the *N*-methyl amide.

The reaction of proline or azetidine derivatives **1b**, **2a**, **3b**, and H-Ala-OMe, using BOP as coupling reagent, led to dipeptides **5b**, **6a**, and **7b**, in good yield, while dipeptide **5a** was purchased from commercial sources. Since the starting azetidine (*R*,*S*)-**3b** is racemic, dipeptide (*R*,*S*)-**7b** was obtained as a mixture of two diastereoisomers which could not be separated. Following approach A the subsequent treatment of esters **5a** and **5b** with $MeNH_2$·EtOH afforded model peptides **8a** and **8b** in good yield. On the contrary, a similar reaction of dipeptide (*R*,*S*)-**7b** led to compound **9b** in low yield (14%) due to the formation of an unexpected product that will be discussed later in Scheme 2. It is interesting to note that only one diastereoisomer of **9b** was obtained from the diastereoisomeric mixture (*R*,*S*)-**7b**. This isomer was identified as the one having 2(*S*)-configuration at the C-2 position of the azetidine ring by comparison with an authentic sample of compound Z-2(*S*)-MeAze-Ala-NHMe (**9b**), prepared from enantiomerically pure Z-2(*S*)-MeAze-OH.[23]

Following the alternative approach B, dipeptide **6a** was deprotected by treatment with saturated HCl in EtOAc, and then converted into compounds **7a**, **10a**, and **11a** by incorporation of benzyloxycarbonyl, acetyl, or pivaloyl groups, respectively, at *N*-terminus. The conversion of esters **7a**, **10a**, and **11a** into their corresponding amides **9a**, **14a**, and **15a** was achieved again by reaction with $MeNH_2$·EtOH.

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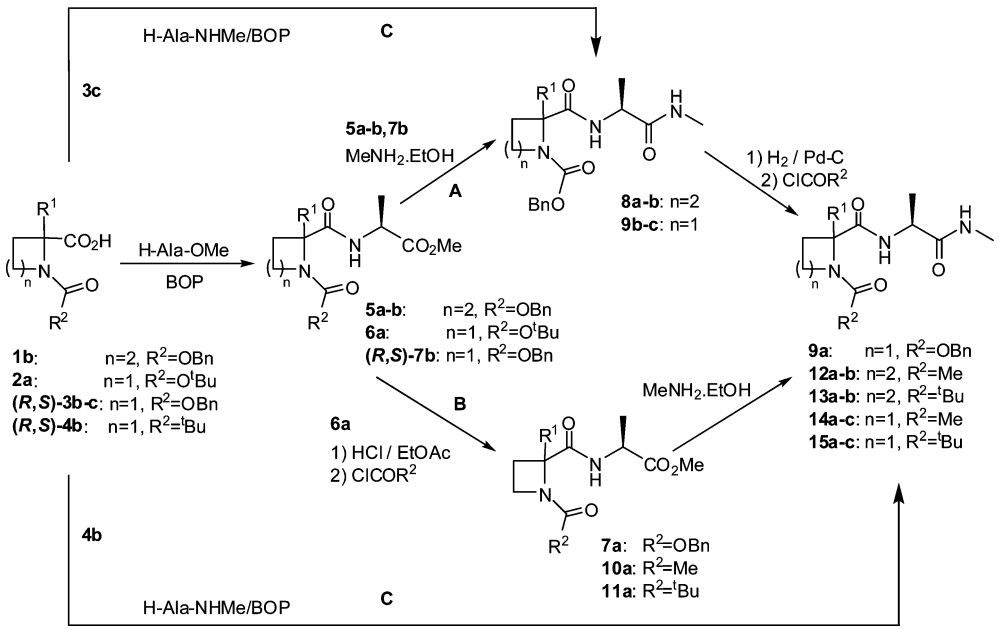
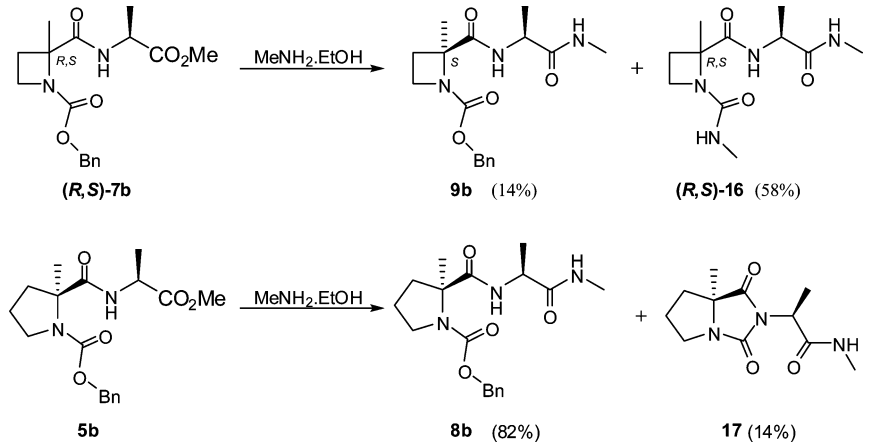
SCHEME 1[a,b]



[a] **a**, R^1 = H; **b**, R^1 = Me; **c**, R^1 = Bn. [b] The stereochemistry on C-2 is (*S*) unless otherwise indicated, except for **9c** and **15b** that are a mixture of two diastereoisomers on C-2 (*R*,*S*).

SCHEME 2



Due to the problems encountered in the aminolysis of (*R*,*S*)-**7b** to **9b**, an alternative route (C) was explored for the preparation of (*R*,*S*)-**9c** and (*R*,*S*)-**15b**. The new method implied the direct coupling of (*R*,*S*)-**3c** and (*R*,*S*)-**4b**, respectively, with H-Ala-NHMe to afford the expected compounds in good yield.[24] Diastereoisomeric dipeptides (*R*,*S*)-**15b** could not be chromatographically resolved, but the comparison of the ^{1}H and ^{13}C NMR of the mixture with those of dipeptide Piv-2(*S*)-MeAze-Ala-NHMe ((*S*)-**15b**), obtained from enantiopure Z-2(*S*)-MeAze-Ala-NHMe (**9b**), allowed the unequivocal assignment of the signals of both diastereoisomers.

Finally, the catalytic hydrogenation of dipeptide derivatives **8a**,**b** and **9b**,**c** using Pd−C as catalyst, followed by *N*-acylation with acetyl or pivaloyl chloride in the presence of TEA resulted in the isolation of compounds **12a**,**b**, **13a**,**b**, **14b**,**c**, and **15b**,**c**. The yield of the latter reactions varied according to the R^2 group, from moderate to good for the acylation with pivaloyl chloride, to low for the reactions with acetyl chloride.

As previously mentioned, the reaction of 2(*R*,*S*)-Z-MeAze-Ala-OMe ((*R*,*S*)-**7b**) with methylamine resulted in a major unexpected product, which was identified as the urea derivative (*R*,*S*)-**16** (Scheme 2), a diastereoisomeric mixture that could not be resolved. Compound (*R*,*S*)-**16** is the result of the nucleophilic intermolecular attack of the nitrogen of the methylamine to the carbonyl group of the benzyloxycarbonyl moiety of (*R*,*S*)-**9b**. This nucleophilic attack should occur with kinetic resolution, since diastereoisomer (*R*)-**9b** was completely converted into (*R*)-**16**, whereas (*S*)-**9b** was only partially transformed. It is worth noticing that in similar reactions with Z-protected dipeptides containing Aze or Pro the formation of the urea secondary product was not appreciated, even after long reaction periods. The only exception was the aminolysis of Z-α-MePro-Ala-OMe (**5b**) with methylamine, which afforded, along with the expected compound **8b**, a secondary product identified as the tetrahydro-1,3-dioxopyrrolo[1,2-*e*]imidazole **17** (14%). Among the ^{1}H and ^{13}C NMR experiments carried out with **17**, the analysis of the bidimensional HMBC spectrum, showing correlations between the α Ala proton and the three carbonyl groups of the molecule, was essential to establish the indicated bicyclic structure.

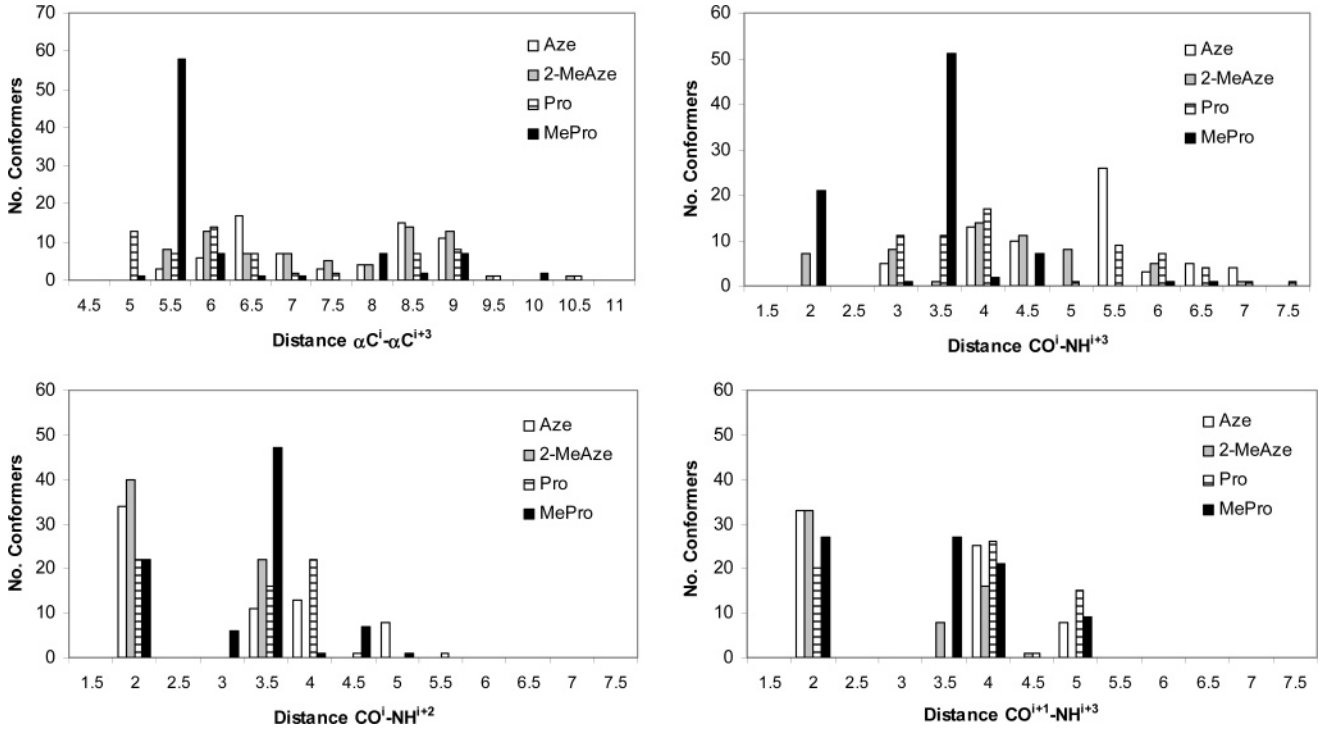


FIGURE 1. Distance distribution (Å) corresponding to the $\alpha C^{i}-\alpha C^{i+3}$ and intramolecular H-bonds for Ac-Xaa-Ala-NHMe trans conformers (Xaa as indicated) within a +3 kcal/mol window of the global minimum.

Molecular Modeling. Theoretical studies were performed with the simplest dipeptide derivatives Ac-Xaa-Ala-NHMe (Xaa = Pro, α-MePro, Aze, 2-MeAze). The conformational studies on these dipeptides were carried out using AMBER as the force field, implemented in InsightII (version 2000.1, Byosym Tech, San Diego, CA). To carry out the molecular dynamic studies, different initial conformations of each peptide were used. The different conformers obtained within +3 kcal mol^{-1} from the global minimum were then grouped into families according to the torsion angles of the peptide skeleton (ϕ and ψ). The dihedral angle (ω) of the *N*-terminal amide was analyzed to study the possible presence of cis/trans isomerism around this bond. In all cases, the cis conformers were shown to have higher energy compared to the trans isomers. Next, the minimum energy conformations with an *N*-terminal trans amide bond were evaluated for their turn propensity by analyzing a series of characteristic parameters of β- and γ-turns. In this particular case, the distances between the α-carbon of the first residue (in our model the CH_3 of the *N*-terminal acetyl group) and the fourth residue (N-CH_3 at *C*-terminal) ($\alpha C^{i}-\alpha C^{i+3} < 7$ Å) and the distance between the oxygen of the carbonyl group of the first residue and the amide proton of the fourth ($CO^{i}-NH^{i+3} \leq 2.5$ Å) were measured to study the possible existence of β-turns in these simple peptide models. On the other hand, the distances between the carbonyl oxygen of the first residue and the amide proton of the third one ($CO^{i}-NH^{i+2} \leq 2.5$ Å) and between the carbonyl oxygen of the second residue and the NH of the fourth ($CO^{i+1}-NH^{i+3} \leq 2.5$ Å) were analyzed to study the possible existence of γ-turns. Finally, for the classification of the type of β- and γ-turns the values of the torsion angles of central residues of the turn were also measured (see Tables 1-4 within the Supporting Information for details).

The values of the characteristic distances indicated above are collected in the histograms depicted in Figure 1. These data

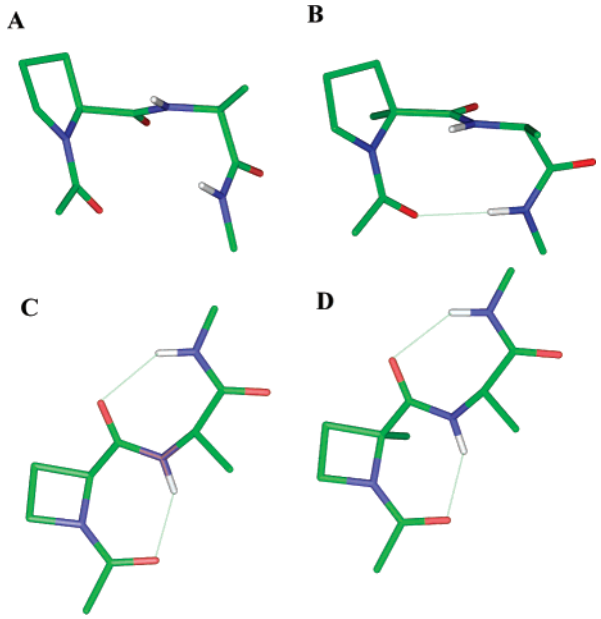


FIGURE 2. Minimal energy conformers of dipeptides MeCO-Xaa-Ala-NHMe (A, Xaa = Pro; B,: Xaa = α-MePro; C, Xaa = Aze; D, Xaa = 2-MeAze).

showed that about 40% of conformers of the Pro-containing dipeptide **12a** fulfill the required $\alpha C^{i}-\alpha C^{i+3}$ distance of β-turn-like conformations, although none of them have the characteristic $CO^{i}-NH^{i+3}$ H-bond. In this case the minimum energy conformer corresponds to an open β-turn (Figure 2A). Dipeptide derivatives with an α-MePro residue have the highest tendency to adopt β-turn conformations. In fact, more than 70% of the conformers showed values of the distance between $\alpha C^{i}-\alpha C^{i+3}$

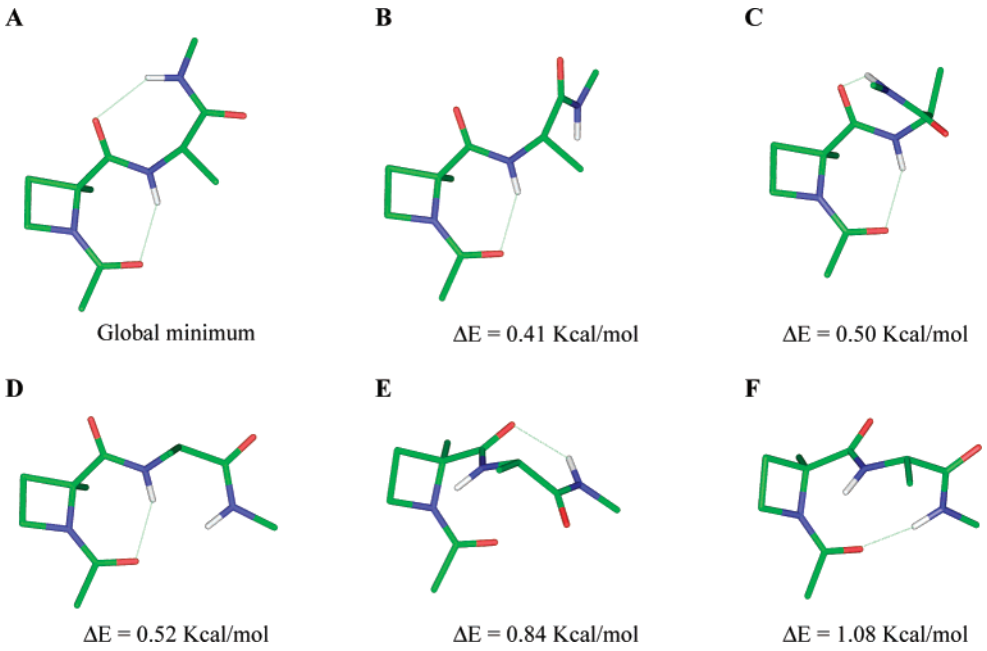


FIGURE 3. Representative conformers for the model tetrapeptide MeCO-2-MeAze-Ala-NHMe (**14b**).

and of the pseudodihedral angle τ within those expected for β-turns, and around 20% of them have the expected $CO^{i}-NH^{i+3}$ H-bond of this reverse turn type. The minimum energy conformer of the α-MePro derivative **12b** is a hydrogen-bonded type I β-turn (Figure 2B), while some other families are stabilized type II β-turns. It is interesting to note that molecular modeling studies also predict a significant number of γ-turn-like conformations for Pro- and α-MePro-containing dipeptides (40% and 30%, respectively).

Azetidine-containing dipeptides showed lower tendency to adopt β-turn-like conformations than Pro analogues, but a high propensity to γ-turn arrangements. Thus, more than 40% of the Ac-Aze-Ala-NHMe (**14a**) conformers have a $CO^{i}-NH^{i+2}$ H-bond, characteristic of a γ-turn centered at the azetidine residue. This percentage increase to 50% for the 2,2-disubstituted azetidine derivative **14b**, with the most frequent H-bond between i and $i + 2$ residues, which correspond to an inverse γ-turn (ϕ^2 around −68°). In both cases, the molecular modeling studies also suggested the possible existence of another γ-turn between $i + 1$ and $i + 3$ residues. In fact, the global minima of compounds **14a** and **14b** show two consecutive γ-turns centered at the second and third residues of the simplified model tetrapeptides (Figure 2C,D). Finally, for the model peptide Ac-2-MeAze-Ala-NHMe (**14b**) there is a family of conformers, 1 kcal/mol above the global minimum, which show the characteristic $CO^{i}-NH^{i+3}$ H-bond and dihedral angles of a type I β-turn (Figure 3F). As can be appreciated in Figure 3, the *C*-terminal amino acid of compound **14b** shows a remarkable conformational flexibility while keeping a γ-turn-like structure at the *N*-terminus in most cases. This is in agreement with previous conformational energy calculations that indicated a larger number of low-energy conformers, and hence a higher flexibility, for Aze-containing dipeptides and Aze-copolymers than for the corresponding Pro analogues.[20,21]

In general, the molecular modeling analysis reported here suggests that Pro derivatives, especially α-MePro, are effective as β-turn inducers, whereas the lower azetidine homologues are more predisposed to induce γ-turns. However, as γ-turn conformations are normally overestimated in theoretical calculations, it is necessary to undertake alternative studies to elucidate if the conformational behavior imposed by Pro and azetidine derivatives when incorporated into peptides is so different as suggested by the molecular modeling study or not.

Conformational Studies in Solution. To experimentally validate whether the theoretically predicted reverse turns are still conserved in solution, the conformational behavior of model peptides **8**, **9**, and **12**−**15** was analyzed using FT-IR and NMR spectroscopy. First, the FT-IR spectra were examined for H-bonded NH stretch bands, which are generally observed at about 3300−3380 cm^{-1}, while free NH bands should appear between 3410 and 3460 cm^{-1}.[27−29] FT-IR experiments were carried out at concentrations of 2 and 20 mM, using $CHCl_3$ as solvent. The form and frequency of the observed bands were independent of the concentration used, thus excluding intermolecular aggregation effects, and indicating that if the H-bonded NH stretch band appears it should be due to intramolecular hydrogen bonds. All dipeptides display non-hydrogen- (3410−3456 cm^{-1}) and hydrogen-bonded states (3346−3377 cm^{-1}), suggesting a certain contribution of H-bonded species (Figure 4). The intensity of the absorption bands corresponding to the NH involved in H-bonds was lower in the Z-protected dipeptides with respect to acetyl and pivaloyl analogues. A greater extent of intramolecular H-bonding was also detected for α-MePro compared to Pro, and for 2-MeAze with respect to Aze (Figure 4), which is consistent with the larger number of H-bonded conformers for the α-alkylated derivatives predicted by the molecular modeling studies.

Further information on the conformational preferences of dipeptides **8**, **9**, and **12**−**15** in solution was obtained from ^1H NMR experiments. In most cases, two distinct sets of signals

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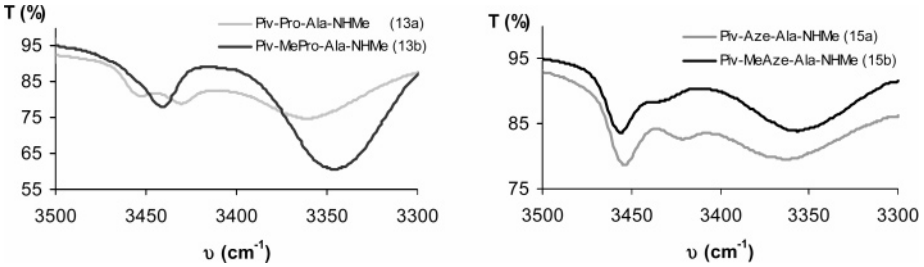
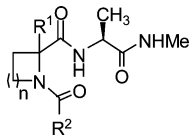


FIGURE 4. NH stretch region of FT-IR spectra of Piv-Xaa-Ala-NHMe at room temperature.

TABLE 1. Ratio of Cis/Trans Conformers in Compounds 8a−15c



compd	*n*	R^1	R^2	% cis ($CDCl_3$)	% cis (DMSO)
8a	2	H	OBn	28	54
12a			Me	14	29
13a			^{t}Bu	0	0
8b		Me	OBn	10	20
12b			Me	0	0
13b			^{t}Bu	0	0
9a	1	H	OBn	0	<5
14a			Me	<5	43
15a			^{t}Bu	<5	<5
9b		Me	OBn	17	43
14b			Me	<5	23
(*S*)-**15b**			^{t}Bu	0	0
(*R*)-**15b**			^{t}Bu	0	0
(*S*)-**9c**		Bn	OBn	<5	31
(*R*)-**9c**			OBn	<5	26
14c			Me	0	0
15c			^{t}Bu	0	0

were observed in the one-dimensional ^{1}H spectra, indicating the existence of cis/trans isomers around the amide bond RCO−Xaa (Xaa = Pro, α-MePro, Aze, 2-MeAze, 2-BnAze).[19,20,28,30,31] The assignment of the cis/trans conformations was made considering a series of criteria that have been widely applied for Xaa-Pro dipeptides:[4,28,30,31] (i) the NOE effect between protons of the R^2−CO moiety and the α-H (Pro) or H-2 (Aze) (cis isomer), and δ-H (Pro) or H-4 (Aze) protons (trans isomer) and (ii) the anisotropic effect of the R^2−CO carbonyl group upon the α-Pro or 2-Aze protons and carbons (trans isomer) or δ-Pro or 4-Aze protons and carbons (cis isomer). Depending on the ^{1}H NMR data, one or more of these criteria were applied for our derivatives. Since for dipeptides **9a** and **9c** none of these criteria could be applied, the mayor isomer was tentatively assigned as trans, considering the high preference toward this rotamer in the other derivatives.

The cis/trans relative populations for the different peptides were determined by integration of separated signals in each ^{1}H NMR spectrum (Table 1). As expected, the population of the less abundant species, cis conformers, increased with the polarity of the solvent, in agreement with previous reports on related Pro derivatives.[32] On the other hand, the percentage of trans conformations rose, in general, with the volume of the substituent attached at position 2 of the azetidine or proline ring, likely due to steric clashes of the alkyl groups at this position with the corresponding residue at the *N*-terminal, which destabilizes the cis isomer. On the contrary, dipeptide derivatives with a Z group at *N*-terminus showed greater population of cis rotamer than the corresponding dipeptides with an acetyl or pivaloyl moiety at this position. Although it has been shown that an aromatic aminoacid *N*-terminal to Pro derivatives increased the cis isomer population through the interaction between the $\delta(+)$ nitrogen of Pro and the aromatic ring of the preceding amino acid,[33,34] molecular dynamic studies on dipeptide derivatives Z-Xaa-Ala-NHMe (Xaa = Pro, α-MePro, Aze, 2-MeAze) have shown that this interaction is not present within the minimum energy conformers (+3 kcal/mol window from the global minimum, data not shown). These theoretical studies have suggested other possible extra stabilizations of the cis conformers: one through an intramolecular H-bond between the Ala amide NH of the dipeptide and the sp^3 oxygen of the urethane group,[35,36] a second one due to an H-bond between the Ala amide NH and the aromatic ring of the Z group,[37] and finally an interaction between the oxygen of the CO (Ala) and the edge of the Z aromatic group.[38,39]

With the only exception of compound **15a**, the ^{1}H NMR spectra of pivaloyl dipeptide derivatives exhibited only one set of resonances, the trans conformation, independent of the *N*-terminal amino acid (Pro or Aze derivative) and the polarity of the solvent used for the NMR analysis.

To explore whether a reverse turn structure was present in model peptide derivatives, we examined the possible involvement of the amide NH groups of compounds **8**, **9**, and **12**−**15** in the intramolecular H-bonds characteristic of β- and γ-turns. The solvent and temperature dependence of the ^{1}H NMR chemical shifts of amide protons evidence their hydrogen-bonding state. It is described that values of the chemical shifts above 7 ppm in $CDCl_3$, together with small variations of these shifts when the solvent is changed to DMSO, are indicative of a possible participation of the corresponding amide proton in an H-bond.[6,27] Application of these criteria to our compounds indicates that the *C*-terminal NHMe proton of α-MePro-

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TABLE 2. Chemical Shifts and Temperature Coefficients for the NH Amide Protons of Compounds 8, 9, and 12−15.

compd	no.	δNH (Ala, ppm)			δNH−Me (ppm)			$\Delta\delta/\Delta T$[a] NH (Ala)	$\Delta\delta/\Delta T$[a] NH−Me
		$CDCl_3$	DMSO	$\Delta\delta_{DMSO-CDCl_3}$	$CDCl_3$	DMSO	$\Delta\delta_{DMSO-CDCl_3}$		
Z-Pro-Ala-NHMe	**8a**	6.95	8.02	1.07	6.86	7.71	0.85	−6.0	−6.3
Ac-Pro-Ala-NHMe	**12a**	7.09	7.88	0.79	6.53	7.56	1.03	−5.0	−3.5
Piv-Pro-Ala-NHMe	**13a**	6.67	7.72	1.05	6.90	7.64	0.74	−5.3	−4.3
Z-α-MePro-Ala-NHMe	**8b**	6.23	7.91	1.68	7.02	7.25	0.23	−6.5	nd
Ac-α-MePro-Ala-NHMe	**12b**	6.44	7.76	1.32	7.04	7.50	0.46	−5.1	−2.2
Piv-α-MePro-Ala-NHMe	**13b**	5.71	7.28	1.57	7.22	7.46	0.24	−5.9	−2.1
Z-Aze-Ala-NHMe	**9a**	7.26	8.09	0.83	6.71	7.78	1.07	−5.3	−4.9
Ac-Aze-Ala-NHMe	**14a**	7.91	8.13	0.32	6.79	7.74	1.05	−3.9	−4.3
Piv-Aze-Ala-NHMe	**15a**	7.60	7.99	0.39	6.80	7.75	0.95	−4.1	−4.6
Z-2-MeAze-Ala-NHMe	**9b**	7.74	7.99	0.25	6.38	7.75	1.37	−4.9	−4.9
Ac-2-MeAze-Ala-NHMe	**14b**	8.51	8.35	0.16	6.44	7.72	1.28	−3.3	−4.4
Piv-2-MeAze-Ala-NHMe	(*S*)-**15b**	8.18	8.07	0.11	6.55	7.71	1.16	−2.7	−4.0
Piv-2-MeAze-Ala-NHMe	(*R*)-**15b**	7.88	7.95	0.07	6.72	7.82	1.10	−2.7	−4.4
Z-2-BnAze-Ala-NHMe	(*S*)-**9c**	8.18	8.22	0.04	6.36	7.81	1.45	−3.0	−5.0
Z-2-BnAze-Ala-NHMe	(*R*)-**9c**	8.22	8.11	0.11	6.31	7.89	1.58	−2.7	−5.4
Ac-2-BnAze-Ala-NHMe	**14c**	8.78	8.67	0.11	6.37	7.78	1.41	−2.8	−4.8
Piv-2-BnAze-Ala-NHMe	**15c**	8.55	8.35	0.20	6.49	7.78	1.29	−1.8	−3.9

[a] Values in ppb/K. Δδ measured in DMSO-d_6, 30−60 °C (each 5 °C for a total of 7 points). nd: not determined.

containing dipeptides and the Ala NH proton of the Aze analogues are solvent-shielded, suggesting their involvement in intramolecular H-bonds (Table 2). Variable-temperature studies, carried out to determine the temperature coefficients of the amide proton, corroborated this assumption. The spectra were recorded between 30 and 60 °C (at 5 deg intervals) in DMSO-d_6, and the temperature coefficients ($\Delta\delta/\Delta T$) were determined from the slopes of the linear regression lines obtained from the chemical shifts versus temperature plots. It is assumed that temperature coefficients equal or less than 3 ppb/K (in absolute value) are indicative of a solvent-shielded NH.[6,27] In small peptides, these data provide information about the NH involvement in an intramolecular hydrogen bond. On the contrary, values above 4 ppb/K show that the NH proton is accessible to the solvent, whereas values of the coefficient in the interval 3−4 ppb/K are not conclusive. The temperature coefficients calculated for the α-MePro-containing peptides **12b** and **13b** were indicative of the existence of an intramolecular H-bond for the NHMe amide proton (Table 2). These figures, in perfect accordance with the results of molecular dynamic simulation described here, and the data reported by other authors,[19] indicate that α-MePro dipeptide derivatives stabilize a β-turn-like conformation in solution. It is worth mentioning the failure of the Pro itself to form the characteristic H-bond of β-turns in these short dipeptides, also in agreement with the results from the molecular modeling studies. In the case of Ac-Pro derivative **12a**, the temperature coefficient for the NHMe amide proton could suggest an open β-turn-like conformation.

Regarding the Aze-containing peptides, we have to distinguish among compounds with azetidine-2-carboxylate and those incorporating 2-methyl- and 2-benzyl-substituted analogues (2-MeAze and 2-BnAze). The NH (Ala) chemical shifts for Aze derivatives **9a**, **14a**, and **15a**, lacking the side chain at position 2, were above 7 ppm in $CDCl_3$, and in **14a** and **15a** there were small variations upon solvent change to DMSO. So, it can be assumed that, at least in the acetyl and pivaloyl derivatives, this NH could be engaged in the formation of an intramolecular H-bond stabilizing a γ-turn-like conformation. However, this assumption was not corroborated by the temperature coefficient values obtained for Aze-containing compounds. On the contrary, most of the dipeptide derivatives incorporating the 2-alkyl-2-carboxy azetidines induce γ-turn-like conformations, as inferred from the chemical shifts (higher than 7 ppm), scarce influence of solvent change ($\Delta\delta_{DMSO-CDCl_3} = 0.04-0.25$), and inaccessibility to solvent ($\Delta\delta/\Delta T < 3$ ppb/K) of the Ala amide NH proton in **9c**, **14b**,**c**, and **15b**,**c** (Table 2). At the same time, the NMR parameters for the *C*-terminal NHMe amide proton of these 2-MeAze and 2-BnAze derivatives did not support the incidence of any significant contribution of β-turn-like conformations. The ability of dipeptide models to adopt a γ-turn conformation centered at the azetidine residue mainly depends on the nature of the *N*-terminal acyl moiety (Piv > Ac > Z) and the 2-alkyl group (Bn > Me ≫ H). It has been suggested for Pro-Xaa dipeptides that an *N*-terminal pivaloyl group could disfavor the γ-turn formation, deduced from the lower chemical shift of the amide proton of the Xaa amino acid in pivaloyl derivatives compared to acetylated compounds.[40] Although this trend in chemical shifts is also followed by our azetidine derivatives, the absolute values of temperature coefficients for model tetrapeptides containing 2-MeAze and 2-BnAze indicated just the contrary, an increased propensity to form γ-turns for Piv-subtituted peptides compared to acetylated derivatives. In short, the difference in chemical shift of amide protons alone should be cautiously taken as an indicator of turn propensity. Finally it is interesting to note that both diastereoisomers of peptides **9c** and **15b** are able to adopt γ-turn-like conformations (Table 2). Therefore, the absolute configuration of the asymmetric carbon of the azetidine ring does not affect the capacity of these non-proteinogenic amino acids to induce reverse turns, but influences the type of γ-turn, inverse for (*S*)-**9c** and (*S*)-**15b**, and classical for (*R*)-**9c** and (*R*)-**15b**.

From our theoretical and experimental conformational studies the general trend of the Aze amino acids to induce γ-turns can be deduced, as well as the positive influence of the α-alkylation upon the capacity to promote this particular conformation. However, previous studies (based on NOESY spectra in CD_2Cl_2) on small linear peptides containing Aze have been interpreted as indicative of the existence of β-turns.[15,20−22] To investigate these divergences NOESY experiments have also been recorded on our model peptides, both in $CDCl_3$ and in DMSO-d_6. The inspection of these spectra showed the existence

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of a weak NOE between the methyl groups at the *N*- and *C*-termini of derivatives with an acetyl or pivaloyl *N*-terminal group (**14a**, **14b**, **15a**, (*S*)-**15b**, **15c**), except for (*S*)-**15b** and **15c** in DMSO-d_6. In the absence of other experimental evidence, this Me−Me NOE might be understood as indicative of β-turn conformations. However, in our case, the chemical shifts and the temperature coefficients of the NH amide protons clearly corroborate a preference of Aze-dipeptides for γ-turn-like arrangements. These discrepancies can be easily reconciled if we consider that several conformations might be present in solution, mainly due to the conformational flexibility of the *C*-terminal residue in R^2CO-R^1Aze-Ala-NHMe dipeptide derivatives (Figure 3). The minimum energy conformers D and F, having γ- and β-turn-like structures, respectively, have distances between the above indicated methyl groups (around 5 Å) that are within the NOE distances. On the other hand, NOE difference studies have been carried out on those signals that can provide clues regarding the population of β-turn-like conformations. A weak NOE was observed between *C*-terminal *N*-Me protons and the *Me*- or *^{t}Bu*-CO for Ac- and Piv-α-MePro-containing peptides, **12b** (0.1%) and **13b** (0.6%), while this NOE was absent for Piv-2-MeAze-Ala-NHMe, (*S*)-**15b**, and only a tiny signal is observed for Ac-2-MeAze-Ala-NHMe, **14b**. In a similar way, a significant NOE between the *C*-terminal NH and the *N*-terminal *Me*- or *^{t}Bu*-groups was exclusively observed for MePro derivatives, **12b** (0.6%) and **13b** (1.1%), but not for Aze analogues. Additionally, medium-strong NOEs between *NH*-Ala and *NH*-Me amide protons were measured for compounds **12b** (2.7%) and **13b (**4.0%), whereas the intensity of these NOEs decreases for Aze-containing peptides **14b** (1.3%) and (*S*)-**15b** (2.1%). Thus, NOE differences are also in agreement with a higher population of β-turn conformations for Pro-containing peptides. On the whole, the experimental data obtained with our peptide models provide evidence that Aze derivatives have a higher tendency than Pro analogues to induce γ-turns, although the conformational flexibility at the *C*-terminus might also allow the adoption of an open β-turn-like arrangement in a much lesser extent.

Conclusions

Theoretical and experimental conformational studies by molecular dynamics, NMR, and IR-FT support that the presence of either Pro or Aze amino acid derivatives in short peptides, RCO-Xaa-Ala-NHMe, biased their conformational preferences toward the adoption of particular reverse turns. The ring size of these $\alpha C^i-\alpha N^i$ cyclized amino acids is crucial to determine the type of reverse turn structure, with the five-membered ring of proline derivatives inducing β-turns, and the four-membered ring of azetidine analogues predisposing the peptides to adopt γ-turn conformations. The incorporation of a substituent at position α plays a decisive role for conferring enough turn inducing capacities to both Pro and Aze derivatives. In conclusion, the 2-alkyl-2-carboxyazetidine amino acids represent a way of stabilizing γ-turn conformations in short peptides, and are a complementary way to proline derivatives for the induction of reverse turns. Thus, they can be used for β- or γ-turn scan, by permutation of the amino acids of peptides of interest by the corresponding proline or azetidine non-proteinogenic analogue, respectively, to provide information regarding the bioactive backbone conformation of these peptides. The 2-alkylazetidine derivatives might open the possibility of achieving suitable templates for the design of biologically active molecules and new foldamers,[41] which could compete for a variety of protein−protein interactions. Further studies are underway in this respect.

Experimental Section

Analytical and spectroscopic data of compounds **14b** and **14c** were described in a preliminary short communication.[23]

General Procedure for the Preparation of RCO-Xaa-Ala-OMe Derivatives. Method A: A solution of the corresponding aminoacid (2.92 mmol) in dry CH_2Cl_2 (8 mL) was treated with H-Ala-OMe·HCl (571 mg, 4.09 mmol), BOP (1.8 g, 4.09 mmol), and TEA (0.97 mL, 7.01 mmol). After being stirred for 24 h at room temperature, the solution was washed successively with citric acid (10%), $NaHCO_3$ (10%), H_2O, and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using the system of eluents indicated in each case.

Method B: Compound **6a** (143 mg, 0.50 mmol) was dissolved in a solution of HCl/EtOAc (12 mL, 3.2 M) and the solution was stirred at room temperature for 4 h. After evaporation of the solvent, the crude was dissolved in dry CH_2Cl_2 (4 mL), cooled at 0 °C, and propylene oxide (0.53 mL, 7.50 mmol) and benzyl chloroformate (0.11 mL, 0.80 mmol) were added. After the solution was stirred for 2 h at room temperature, the organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was washed successively with citric acid (10%), $NaHCO_3$ (10%), H_2O, and brine, and subsequently purified by preparative radial chromatography, using a gradient from 20% to 66% of EtOAc in hexane.

Method C: Compound **6a** (143 mg, 0.50 mmol) was stirred in a solution of HCl/AcOEt (12 mL, 3.2 M) at room temperature for 4 h. After evaporation of the solvent, the crude was dissolved in dry CH_2Cl_2 (4 mL) and cooled at 0 °C under argon atmosphere. To this solution was added TEA (0.22 mL, 1.55 mmol) and the corresponding acyl chloride (0.80 mmol). After being stirred for 2 h at room temperature the solution was washed successively with citric acid (10%), $NaHCO_3$ (10%), H_2O, and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using the system of eluents indicated in each case.

Z-L-α-MePro-L-Ala-OMe (5b): Syrup, 82% yield (from **1b**, Method A). Gradient from 25% to 33% of EtAcO in hexane. HPLC: t_R = 4.69 min (A:B = 30:70). $[\alpha]_D$ −55.58 (*c* 1.46, $CHCl_3$). ^{1}H NMR ($CDCl_3$): cis/trans isomers ratio: 1:1.7. Trans isomer: δ 7.51 (br s, 1H, NH-Ala), 7.30 (m, 5H, Ph), 5.18 (d, 1H, J = 12.5 Hz, CH_2-Z), 5.11 (d, 1H, J = 12.5 Hz, CH_2-Z), 4.46 (m, 1H, α-H, Ala), 3.73 (s, 3H, OMe), 3.60 (m, 2H, δ-H, Pro), 2.64 (m, 1H, β-H, Pro), 1.81 (m, 3H, β-H, γ-H, Pro), 1.68 (s, 3H, α-CH_3, Pro), 1.36 (m, 3H, β-H, Ala). Cis isomer: δ 7.51 (br s, 1H, NH-Ala), 7.30 (m, 5H, Ph), 5.16 (s, 2H, CH_2-Z), 4.46 (m, 1H, α-H, Ala), 3.65 (s, 3H, OMe), 3.60 (m, 2H, δ-H, Pro), 2.27 (m, 1H, β-H, Pro), 1.81 (m, 3H, β-H, γ-H, Pro), 1.47 (s, 3H, α-CH_3, Pro), 1.27 (m, 3H, β-H, Ala). ^{13}C NMR ($CDCl_3$): trans isomer: δ 173.9 (CON), 173.3 (COO), 155.1 (OCON), 136.5 (C-Ph), 128.4, 127.9, 127.7 (CH−Ph), 67.1 (α-C, Pro), 66.9 (CH_2-Z), 52.2 (OMe), 48.5 (δ-C, Pro), 48.3 (α-C, Ala), 38.7 (β-C, Pro), 22.6 (γ-C, Pro), 22.5 (α-CH_3, Pro), 17.9 (β-C, Ala). ES-MS: 349.2 $[M + 1]^+$, 371.2 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C 62.05, H 6.94, N 8.04. Found: C 62.00, H 6.92, N 8.09.

2(*S*)-Boc-Aze-L-Ala-OMe (6a): Syrup, 93% yield (from **2a**, Method A). Eluent: gradient from 33% to 66% of EtAcO in hexane. $[\alpha]_D$ −117.16 (*c* 1.38, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.40 (m, 1H, NH-Ala), 4.66−4.53 (m, 2H, α-H, Ala, H-2), 3.84 (m, 2H, H-4), 3.72 (s, 3H, OMe), 2.40 (m, 2H, H-3), 1.44 (s, 9H, CH_3-^{t}Bu), 1.41 (d, 3H, J = 7.1 Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): δ 173.0 (COO), 171.2 (CON), 157.1 (OCON), 81.1 (C-^{t}Bu), 62.2 (2-C), 52.4 (OMe),

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48.0 (α-C, Ala), 47.4 (4-C), 28.3 (CH_3-^{t}Bu), 19.9 (3-C), 18.4 (β-C, Ala). ES-MS: 309.2 $[M + Na]^+$, 595.2 $[2M + Na]^+$. Anal. Calcd for $C_{13}H_{22}N_2O_5$: C 54.53, H 7.74, N 9.78. Found: C 54.47, H 7.80, N 9.85.

2(*S*)-Z-Aze-L-Ala-OMe (7a). Syrup, 61% yield (Method B). Eluent: gradient from 20% to 66% of EtAcO in hexane. HPLC: t_R = 8.59 min (A:B = 20:80). $[\alpha]_D$ −77.98 (*c* 1.06, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.35 (m, 6H, NH-Ala, Ph), 5.18 (d, 1H, *J* = 12.2 Hz, CH_2-Z), 5.12 (d, 1H, *J* = 12.2 Hz, CH_2-Z), 4.73 (t, 1H, *J* = 8.0 Hz, H-2), 4.56 (q, 1H, *J* = 7.2 Hz, α-H, Ala), 3.95 (m, 2H, H-4), 3.73 (s, 3H, OMe), 2.47 (m, 2H, H-3), 1.39 (d, 3H, *J* = 7.2 Hz, β-H, Ala). ^{1}H NMR (DMSO-d_6): δ 8.40 (m, 1H, NH-Ala), 7.32 (m, 5H, Ph), 5.00 (m, 2H, CH_2-Z), 4.60 (dd, 1H, *J* = 8.7, 5.1 Hz, H-2), 4.28 (q, 1H, *J* = 7.5 Hz, α-H, Ala), 3.88 (m, 2H, H-4), 3.60 (s, 3H, OMe), 2.44 (m, 1H, H-3), 2.00 (m, 1H, H-3), 1.24 (d, 3H, *J* = 7.5 Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): δ 172.7 (COO), 170.5 (CON), 157.3 (OCON), 135.9 (C-Ph), 128.4, 128.1, 127.9 (CH-Ph), 67.1 (CH_2-Z), 62.1 (2-C), 52.2 (OMe), 47.9 (α-C, Ala), 47.2 (4-C), 19.9 (3-C), 18.0 (β-C, Ala). ES-MS: 321.2 $[M + 1]^+$, 343.2 $[M + Na]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_5$: C 59.99, H 6.29, N 8.74. Found: C 60.03, H 6.21, N 8.62.

2(*R,S*)-Z-MeAze-L-Ala-OMe ((*R,S*)-7b): Syrup, 89% yield (from (*R,S*)-**3b**, Method A). Eluent: MeOH:CH_2Cl_2 (1:90). HPLC: t_R = 8.30 min (A:B = 25:75). ^{1}H NMR ($CDCl_3$): δ 8.23−7.98 (br s, 1H, NH-Ala), 7.37 (m, 5H, Ph), 5.17 (br s, 2H, CH_2-Z), 4.53 (q, 1H, *J* = 7.2 Hz, α-H, Ala), 4.00−3.60 (m, 2H, H-4), 3.75 (s, 3H, OMe), 2.80−2.44 (m, 1H, H-3), 2.05 (m, 1H, H-3), 1.80−1.56 (br s, 3H, 2-CH_3), 1.50−1.15 (m, 3H, β-H, Ala). ^{13}C NMR ($CDCl_3$): diastereoisomer A: δ 174.1 (CON), 173.0 (COO), 156.7 (OCON), 136.1 (C-Ph), 128.5, 128.1, 127.9 (CH-Ph), 70.0 (1-C), 66.9 (CH_2-Z), 52.2 (OMe), 48.0 (α-C, Ala), 44.4 (4-C), 27.3 (3-C), 22.5 (2-CH_3), 17.8 (β-H, Ala); diastereoisomer B: δ 174.1 (CON), 173.0 (COO), 156.7 (OCON), 136.1 (C-Ph), 128.5, 128.1, 127.9 (CH-Ph), 70.0 (1-C), 66.9 (CH_2-Z), 52.2 (OMe), 48.0 (α-C, Ala), 44.4 (4-C), 27.6 (3-C), 22.5 (2-CH_3), 17.8 (β-C, Ala). ES-MS: 335.2 $[M + 1]^+$, 357.2 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{22}N_2O_5$: C 61.07, H 6.63, N 8.38. Found: C 61.27, H 6.60, N 8.47.

2(*S*)-Ac-Aze-L-Ala-OMe (10a): Oil, 79% yield (Method C). Eluent: MeOH:CH_2Cl_2 (1:10) (centrifugal circular thin-layer chromatography). $[\alpha]_D$ −119.28 (*c* 0.52, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.28 (m, 1H, NH-Ala), 4.88 (dd, 1H, *J* = 9.5, 6.8 Hz, H-2), 4.50 (q, 1H, *J* = 7.1 Hz, α-H, Ala), 4.06 (t, 2H, *J* = 7.8 Hz, H-4), 3.73 (s, 3H, OMe), 2.70 (m, 1H, H-3), 2.45 (m, 1H, H-3), 1.92 (s, 3H, CH_3-CO), 1.41 (d, 3H, *J* = 7.1 Hz, β-H, Ala). ^{1}H NMR (DMSO-d_6): cis/trans isomers ratio: 1:1. Isomer A: δ 8.58 (d, 1H, *J* = 7.1 Hz, NH-Ala), 4.78 (m, 1H, H-2), 4.35 (q, 1H, *J* = 7.1 Hz, α-H, Ala), 4.02 (t, 2H, *J* = 7.6 Hz, H-4), 3.30 (s, 3H, OMe), 2.36 (m, 1H, H-3), 2.02 (m, 1H, H-3), 1.76 (s, 3H, CH_3-CO), 1.30 (d, 3H, *J* = 7.1 Hz, β-H, Ala); isomer B: δ 8.37 (d, 1H, *J* = 7.1 Hz, NH-Ala), 4.59 (m, 1H, H-2), 4.27 (q, 1H, *J* = 7.1 Hz, α-H, Ala), 3.75 (t, 2H, *J* = 7.2 Hz, H-4), 3.30 (s, 3H, OMe), 2.36 (m, 1H, H-3), 2.02 (m, 1H, H-3), 1.64 (s, 3H, CH_3-CO), 1.27 (d, 3H, *J* = 7.1 Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): δ 172.7 (COO), 172.5 (CO-CH_3), 170.3 (CON), 61.6 (2-C), 52.1 (OMe), 50.6 (4-C), 48.5 (α-C, Ala), 18.7 (CH_3-CO), 18.0 (3-C), 17.6 (β-C, Ala). ES-MS: 229.2 $[M + 1]^+$, 251.2 $[M + Na]^+$, 479.2 $[2M + Na]^+$. Anal. Calcd for $C_{10}H_{16}N_2O_4$: C 52.62, H 7.07, N 12.27. Found: C 52.60, H 7.10, N 12.31.

2(*S*)-Piv-Aze-L-Ala-OMe (11a): Syrup, 82% yield (Method C). Eluent: MeOH:CH_2Cl_2 (1:10) (centrifugal circular thin-layer chromatography). $[\alpha]_D$ −98.38 (*c* 0.62, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.87 (m, 1H, NH-Ala), 4.82 (dd, 1H, *J* = 9.5, 6.0 Hz, H-2), 4.41 (q, 1H, *J* = 7.2 Hz, α-H, Ala), 4.26 (t, 2H, *J* = 8.4 Hz, H-4), 3.64 (s, 3H, OMe), 2.51 (m, 1H, H-3), 2.35 (m, 1H, H-3), 1.30 (d, 3H, *J* = 7.2 Hz, β-H, Ala), 1.12 (s, 9H, CH_3-^{t}Bu). ^{1}H NMR (DMSO-d_6): δ 8.31 (br s, 1H, NH-Ala), 4.60 (m, 1H, H-2), 4.42−4.05 (m, 3H, α-H, Ala, H-4), 3.62 (s, 3H, OMe), 2.39 (m, 1H, H-3), 1.95 (m, 1H, H-3), 1.27 (d, 3H, *J* = 7.2 Hz, β-H, Ala), 1.08 (s, 9H, CH_3-^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 179.6 (CO-^{t}Bu), 172.8 (COO), 170.5 (CON), 62.4 (2-C), 52.1 (OMe), 51.7 (4-C), 48.0 (α-C, Ala), 38.4 (C-^{t}Bu), 26.8 (CH_3-^{t}Bu), 18.8 (3-C), 17.7 (β-C, Ala). ES-MS: 271.3 $[M + 1]^+$, 293.2 $[M + Na]^+$, 563.3 $[2M + Na]^+$. Anal. Calcd for $C_{13}H_{22}N_2O_4$: C 57.76, H 8.20, N 10.36. Found: C 57.88, H 8.12, N 10.41.

General Procedures for the Preparation of RCO-Xaa-Ala-NHMe Derivatives. Method A: The corresponding ester (4.22 mmol) was dissolved in $MeNH_2$·EtOH (10 mL, 8M) and reacted over 2−12 h at room temperature. When necessary, the reaction was purified by flash chromatography or centrifugal circular thin-layer chromatography, using the system of eluents indicated in each case.

Method B: The corresponding azetidine (2.92 mmol) was dissolved in dry CH_2Cl_2 (8 mL) and treated with H-Ala-NHMe·HCl (567 mg, 4.09 mmol), BOP (1.81 g, 4.09 mmol), and TEA (0.97 mL, 7.01 mmol). After being stirred for 24 h at room temperature, the solution was washed successively with citric acid (10%), $NaHCO_3$ (10%), H_2O, and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using as eluent EtOAc:hexane (1:4).

Method C: To a solution of the corresponding Z-protected dipeptide NHMe amide (0.34 mmol) in MeOH (13 mL), at 0 °C, was added 10% Pd−C (15% w/w) and the solution was hydrogenated at 15 psi and room temperature. After 1 h the catalyst was filtered and the solvent was evaporated to dryness. The resulting residue was dissolved in dry CH_2Cl_2 (4 mL), cooled to 0 °C under argon atmosphere, and treated with TEA (0.15 mL, 1.05 mmol) and the corresponding acyl chloride (0.54 mmol). After being stirred for 2 h at room temperature, the solution was washed successively with citric acid (10%), $NaHCO_3$ (10%), H_2O, and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography, using the system of eluents indicated in each case.

Z-L-Pro-L-Ala-NHMe (8a):[42] Solid (mp 129−131 °C), 100% yield (from **5a**, Method A). HPLC: t_R= 9.55 min (trans isomer) and 8.21 min (cis isomer) (A:B = 15:85). $[\alpha]_D$ −89.12 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): cis/trans isomers ratio: 1:2.6. Trans isomer: δ 7.25 (m, 5H, Ph), 6.95 (d, 1H, *J* = 7.2 Hz, NH-Ala), 6.86 (br s, 1H, NH-CH_3), 5.05 (br s, 2H, CH_2-Z), 4.38 (q, 1H, *J* = 7.2 Hz, α-H, Ala), 4.25 (m, 1H, α-H, Pro), 3.45 (m, 2H, δ-H, Pro), 2.65 (m, 3H, CH_3-NH), 2.04 (m, 2H, β-H, Pro), 1.83 (m, 2H, γ-H, Pro), 1.25 (d, 3H, *J* = 7.2 Hz, β-H, Ala); cis isomer: δ 7.25 (m, 5H, Ph), 6.95 (m, 1H, NH-Ala), 6.86 (br s, 1H, NH-CH_3), 5.05 (br s, 2H, CH_2-Z), 4.38 (m, 1H, α-H, Ala), 4.25 (m, 1H, α-H, Pro), 3.45 (m, 2H, δ-H, Pro), 2.58 (br s, 3H, CH_3-NH), 2.04 (m, 2H, β-H, Pro), 1.83 (m, 2H, γ-H, Pro), 1.15 (br s, 3H, β-H, Ala). ^{1}H NMR (DMSO-d_6): cis/trans isomers ratio: 1:1.15. Trans isomer: δ 8.02 (m, 1H, NH-Ala), 7.71 (m, 1H, NH-CH_3), 7.33 (m, 5H, Ph), 5.06 (d, 1H, *J* = 12.8 Hz, CH_2-Z), 4.94 (d, 1H, *J* = 12.8 Hz, CH_2-Z), 4.25 (m, 1H, α-H, Pro), 4.19 (q, 1H, *J* = 7.2 Hz, α-H, Ala), 3.40 (m, 2H, δ-H, Pro), 2.55 (br s, 3H, CH_3-NH), 2.10 (m, 1H, β-H, Pro), 1.82 (m, 3H, β-H, Pro, γ-H, Pro), 1.06 (d, 3H, *J* = 7.2 Hz, β-H, Ala); cis isomer: δ 8.02 (m, 1H, NH-Ala), 7.62 (m, 1H, NH−CH_3), 7.33 (m, 5H, Ph), 5.05 (s, 2H, CH_2-Z), 4.19 (m, 1H, *J* = 7.2 Hz, α-H, Ala), 4.17 (m, 1H, α-H, Pro), 3.40 (m, 2H, δ-H, Pro), 2.55 (br s, 3H, CH_3-NH), 2.10 (m, 1H, β-H, Pro), 1.82 (m, 3H, β-H, Pro, γ-H, Pro), 1.18 (d, 3H, *J* = 7.2 Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): trans isomer: δ 172.5 and 171.6 (CON), 155.8 (OCON), 136.1 (C-Ph), 128.3, 128.0, 127.6 (CH-Ph), 67.2 (CH_2-Z), 60.8 (α-C, Pro), 48.7 (α-C, Ala), 47.0 (δ-C, Pro), 29.3 (β-C, Pro), 26.0 (CH_3-NH), 24.4 (γ-C, Pro), 17.8 (β-C, Ala); cis isomer: δ 172.1 and 171.6 (CON), 154.6 (OCON), 136.1 (C-Ph), 128.3, 128.2, 127.6 (CH-Ph), 67.2 (CH_2-Z), 60.4 (α-C, Pro), 48.7 (α-C, Ala), 47.3 (δ-C, Pro), 31.1 (β-C, Pro), 26.0 (CH_3-NH), 23.5 (γ-C, Pro), 18.2

(42) Partial characterization details can be found in: Ohler, E.; Schmidt, U. *Chem. Ber.* **1977**, *110*, 921−941.

(β-C, Ala). ES-MS: 334.0 $[M + 1]^+$, 356.0 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{23}N_3O_4$: C 61.25, H 6.95, N 12.60. Found: C 61.44, H 6.88, N 12.80.

Z-L-α-MePro-L-Ala-NHMe (8b): Syrup, 82% yield (from **5b**, Method A). Eluent: MeOH:CH_2Cl_2 (1:20). HPLC: t_R = 11.47 min (A:B = 20:80). $[\alpha]_D$ −31.14 (*c* 0.97, $CHCl_3$). ^{1}H NMR ($CDCl_3$): cis/trans isomers ratio: 1:6.1. Trans isomer: δ 7.34 (m, 5H, Ph), 7.02 (br s, 1H, NH-CH_3), 6.23 (d, 1H, J = 7.4 Hz, NH-Ala), 5.18 (d, 1H, J = 12.4 Hz, CH_2-Z), 5.08 (d, 1H, J = 12.4 Hz, CH_2-Z), 4.42 (q, 1H, J = 7.4 Hz, α-H, Ala), 3.63 (m, 2H, δ-H, Pro), 2.71 (d, 3H, J = 4.5 Hz, CH_3-NH), 2.22 (m, 1H, β-H, Pro), 1.98−1.86 (m, 3H, β-H, Pro, γ-H, Pro), 1.58 (s, 3H, α-CH_3, Pro), 1.30 (d, 3H, J = 7.4 Hz, β-H, Ala); cis isomer: δ 7.34 (m, 5H, Ph), 6.44 (br s, 1H, NH-CH_3), 6.23 (d, 2H, J = 7.4 Hz, NH-Ala), 5.17 (s, 2H, CH_2-Z), 4.42 (m, 1H, α-H, Ala), 3.63 (m, 2H, δ-H, Pro), 2.71 (m, 3H, CH_3-NH), 2.22 (m, 1H, β-H, Pro), 1.98−1.86 (m, 3H, β-H, Pro, γ-H, Pro), 1.58 (s, 3H, α-CH_3, Pro), 1.30 (m, 3H, β-H, Ala). ^{1}H NMR (DMSO-d_6): cis/trans isomers ratio: 1:2.9. Trans isomer: δ 7.91 (d, 1H, J = 7.5 Hz, NH-Ala), 7.42−7.20 (m, 6H, NH-CH_3, Ph), 5.12 (d, 1H, J = 12.9 Hz, CH_2-Z), 5.05 (d, 1H, J = 12.9 Hz, CH_2-Z), 4.19 (q, 1H, J = 7.5 Hz, α-H, Ala), 3.63 (m, 1H, δ-H, Pro), 3.51 (m, 1H, δ-H, Pro), 2.51 (d, 3H, J = 4.6 Hz, CH_3-NH), 2.10 (m, 1H, β-H, Pro), 1.95−1.70 (m, 3H, β-H, Pro, γ-H, Pro), 1.40 (s, 3H, α-CH_3, Pro), 1.21 (d, 3H, J = 7.5 Hz, β-H, Ala); cis isomer: δ 7.60 (m, 1H, NH-CH_3), 7.54 (d, 1H, J = 7.4 Hz, NH-Ala), 7.42−7.20 (m, 5H, Ph), 5.05 (d, 1H, J = 12.9 Hz, CH_2-Z), 4.89 (d, 1H, J = 12.9 Hz, CH_2-Z), 4.19 (m, 1H, α-H, Ala), 3.51 (m, 1H, δ-H, Pro), 3.20 (m, 1H, δ-H, Pro), 2.51 (m, 3H, CH_3-NH), 2.10 (m, 1H, β-H, Pro), 1.95−1.70 (m, 3H, β-H, Pro, γ-H, Pro), 1.46 (s, 3H, α-CH_3, Pro), 1.13 (d, 3H, J = 7.4 Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): trans isomer: δ 173.4 and 172.5 (CON), 155.0 (OCON), 136.3 (C-Ph), 128.6, 128.3, 127.7 (CH-Ph), 68.7 (α-C, Pro), 67.3 (CH_2-Z), 49.1 (α-C, Ala), 47.8 (δ-C, Pro), 39.3 (β-C, Pro), 26.2 (CH_3-NH), 23.0 (γ-C, Pro), 21.1 (α-CH_3, Pro), 17.8 (β-C, Ala); cis isomer: δ 173.4 and 172.5 (CON), 155.0 (OCON), 136.3 (C−Ph), 128.6, 128.3, 127.7 (CH-Ph), 68.7 (α-C, Pro), 67.0 (CH_2-Z), 49.1 (α-C, Ala), 47.8 (δ-C, Pro), 39.3 (β-C, Pro), 26.2 (CH_3-NH), 23.0 (γ-C, Pro), 21.1 (α-CH_3, Pro), 17.8 (β-C, Ala). ES-MS: 348.0 $[M + 1]^+$, 370.0 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{25}N_3O_4$: C 62.23, H 7.25, N 12.10. Found: C 62.42, H 7.22, N 12.33.

2(*S*)-Z-Aze-L-Ala-NHMe (9a): White solid (mp 172−174 °C), 99% yield (from **7a**, Method A). HPLC: t_R = 6.93 min (A:B = 15:85). $[\alpha]_D$ −102.25 (*c* 0.40, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.26 (m, 6H, NH-Ala, Ph), 6.71 (m, 1H, NH-CH_3), 5.07 (d, 1H, J = 12.3 Hz, CH_2-Z), 5.01 (d, 1H, J = 12.3 Hz, CH_2-Z), 4.63 (t, 1H, J = 7.7 Hz, H-2), 4.41 (q, 1H, J = 7.0 Hz, α-H, Ala), 3.85 (m, 2H, H-4), 2.65 (d, 3H, J = 4.3 Hz, CH_3-NH), 2.35 (m, 2H, H-3), 1.28 (d, 3H, J = 7.0 Hz, β-H, Ala). ^{1}H NMR (DMSO-d_6): δ 8.09 (d, 1H, J = 7.2 Hz, NH-Ala), 7.78 (m, 1H, NH-CH_3), 7.32 (m, 5H, Ph), 5.01 (m, 2H, CH_2-Z), 4.63 (dd, 1H, J = 8.4, 5.2 Hz, H-2), 4.24 (q, 1H, J = 7.2 Hz, α-H, Ala), 3.87 (m, 2H, H-4), 2.56 (d, 3H, J = 4.6 Hz, CH_3-NH), 2.40 (m, 1H, H-3), 2.04 (m, 1H, H-3), 1.15 (m, 3H, β-H, Ala). ^{13}C NMR ($CDCl_3$): δ 172.3 and 170.7 (CON), 157.3 (OCON), 135.7 (C-Ph), 128.4, 128.2, 127.9 (CH-Ph), 67.2 (CH_2-Z), 62.1 (2-C), 48.7 (α-C, Ala), 47.3 (4-C), 25.8 (CH_3-NH), 20.2 (3-C), 18.0 (β-C, Ala). ES-MS: 320.0 $[M + 1]^+$, 342.0 $[M + Na]^+$. Anal. Calcd for $C_{16}H_{21}N_3O_4$: C 60.17, H 6.63, N 13.16. Found: C 60.26, H 6.44, N 13.06.

2(*S*)-Z-MeAze-L-Ala-NHMe (9b): Syrup, 14% yield (from (*R*,*S*)-**7b**, Method A) and 90% yield (from **3b**, Method B). Eluent: MeOH:CH_2Cl_2 (1:60). HPLC: t_R = 16.03 min (cis isomer) and 18.75 min (trans isomer) (A:B = 15:85). $[\alpha]_D$ −113.13 (*c* 0.98, $CHCl_3$). ^{1}H NMR ($CDCl_3$): cis/trans isomers ratio: 1:5. Trans isomer: δ 7.74 (m, 1H, NH-Ala), 7.30 (m, 5H, Ph), 6.38 (m, 1H, NH-CH_3), 5.08 (s, 2H, CH_2-Z), 4.34 (q, 1H, J = 7.1 Hz, α-H, Ala), 3.89 (m, 1H, H-4), 3.74 (m, 1H, H-4), 2.72 (d, 3H, J = 3.8 Hz, CH_3-NH), 2.60 (m, 1H, H-3), 1.98 (m, 1H, H-3), 1.64 (s, 3H, 2-CH_3), 1.30 (d, 3H, J = 7.1 Hz, β-H, Ala). ^{1}H NMR (DMSO-d_6): cis/trans isomers ratio: 1:1.3. Trans isomer: δ 7.99 (d, 1H, J = 7.1 Hz, NH-Ala), 7.75 (m, 1H, NH-CH_3), 7.35 (m, 5H, Ph), 5.07 (s, 2H, CH_2-Z), 4.23 (q, 1H, J = 7.1 Hz, α-H, Ala), 3.78 (m, 2H, H-4), 2.58 (d, 3H, J = 3.9 Hz, CH_3-NH), 2.33 (m, 1H, H-3), 2.02 (m, 1H, H-3), 1.55 (s, 3H, 2-CH_3), 1.19 (d, 3H, J = 7.1 Hz, β-H, Ala); cis isomer: δ 7.75 (m, 2H, NH-Ala, NH-CH_3), 7.35 (m, 5H, Ph), 5.06 (d, 1H, J = 12.7 Hz, CH_2-Z), 4.99 (d, 1H, J = 12.7 Hz, CH_2-Z), 4.23 (m, 1H, α-H, Ala), 3.92 (m, 2H, H-4), 2.54 (d, 3H, J = 3.9 Hz, CH_3-NH), 2.33 (m, 1H, H-3), 2.02 (m, 1H, H-3), 1.55 (s, 3H, 2-CH_3), 1.19 (m, 3H, β-H, Ala). ^{13}C NMR ($CDCl_3$): δ 174.0 and 172.4 (CON), 156.2 (OCON), 136.0 (C-Ph), 128.5, 128.3, 128.0 (CH-Ph), 69.9 (2-C), 67.1 (CH_2-Z), 48.9 (α-C, Ala), 44.6 (4-C), 29.6 (3-C), 26.2 (CH_3-NH), 22.0 (2-CH_3), 17.9 (β-C, Ala). ES-MS: 335.2 $[M + 1]^+$, 357.2 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{23}N_3O_4$: C 61.25, H 6.95, N 12.60, Found: C 61.08, H 6.77, N 12.79.

2(*S*)-Z-BnAze-L-Ala-NHMe ((*S*)-9c): Solid (mp 132−134 °C), 48% yield (from (*R*,*S*)-**3c**, Method B). Eluent: EtOAc:hexane (1:4). HPLC: t_R = 19.93 min (A:B = 30:70). $[\alpha]_D$ +12.3 (*c* 0.34, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.18 (d, 1H, J = 7.2 Hz, NH-Ala), 7.46−7.07 (m, 10H, Ph), 6.36 (br s, 1H, NH-CH_3), 5.28 (d, 1H, J = 12.2 Hz, CH_2-Z), 5.09 (d, 1H, J = 12.2 Hz, CH_2-Z), 4.42 (q, 1H, J = 7.2 Hz, α-H, Ala), 3.51 (m, 1H, H-4), 3.49 (d, 1H, J = 13.9 Hz, 2-CH_2), 3.02 (d, 1H, J = 13.9 Hz, 2-CH_2), 2.96 (m, 1H, H-4), 2.79 (d, 3H, J = 4.9 Hz, CH_3-NH), 2.60 (m, 1H, H-3), 2.19 (m, 1H, H-3), 1.40 (d, 3H, J = 7.2 Hz, β-H, Ala). ^{1}H NMR (DMSO-d_6): cis/trans isomers ratio: 1:2.25. Trans isomer: δ 8.23 (d, 1H, J = 7.4 Hz, NH-Ala), 7.81 (m, 1H, NH-CH_3), 7.39−7.12 (m, 10H, Ph), 5.24 (d, 1H, J = 12.3 Hz, CH_2-Z), 5.04 (d, 1H, J = 12.3 Hz, CH_2-Z), 4.28 (m, 1H, α-H, Ala), 3.53 (m, 1H, H-4), 3.31 (d, 1H, J = 13.8 Hz, 2-CH_2), 3.00 (d, 1H, J = 13.8 Hz, 2-CH_2), 2.82 (m, 1H, H-4), 2.61 (d, 3H, J = 3.8 Hz, CH_3-NH), 2.32 (m, 1H, H-3), 2.10 (m, 1H, H-3), 1.23 (d, 3H, J = 6.5 Hz, β-H, Ala); cis isomer: δ 7.93 (d, 1H, J = 7.5 Hz, NH-Ala), 7.75 (m, 1H, NH-CH_3), 7.39−7.12 (m, 10H, Ph), 5.24 (m, 2H, CH_2-Z), 4.28 (m, 1H, α-H, Ala), 3.53 (m, 1H, H-4), 3.13 (s, 2H, 2-CH_2), 2.79 (m, 1H, H-4), 2.57 (d, 3H, J = 3.8 Hz, CH_3-NH), 2.32 (m, 1H, H-3), 2.07 (m, 1H, H-3), 1.23 (d, 3H, J = 6.5 Hz, β-H, Ala). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.8 and 172.4 (CON), 156.3 (OCON), 136.0, 134.6 (C-Ph), 130.3, 128.5, 128.3, 127.1 (CH-Ph), 73.3 (2-C), 67.1 (CH_2-Z), 49.1 (α-C-Ala), 44.8 (4-C), 39.0 (2-CH_2), 26.3 (CH_3-NH), 23.6 (3-C), 17.3 (β-C, Ala). ES-MS: 410.2 $[M + 1]^+$, 432.1 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{27}N_3O_4$: C 67.46, H 6.65, N 10.26, Found: C 67.22, H 6.84, N 10.01.

2(*R*)-Z-BnAze-L-Ala-NHMe ((*R*)-9c): Foam, 12% yield (from (*R*,*S*)-**3c**, Method B). Eluent: EtOAc:hexane (1:4). HPLC: t_R = 17.56 min (A:B = 30:70). $[\alpha]_D$ −40.2 (*c* 0.31, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.22 (d, 1H, J = 7.0 Hz, NH-Ala), 7.45−7.10 (m, 10H, Ph), 6.31 (br s, 1H, NH-CH_3), 5.33 (d, 1H, J = 12.1 Hz, CH_2-Z), 5.04 (d, 1H, J = 12.1 Hz, CH_2-Z), 4.43 (q, 1H, J = 7.0 Hz, α-H, Ala), 3.51 (d, 1H, J = 13.9 Hz, 2-CH_2), 3.49 (m, 1H, H-4), 2.94 (d, 1H, J = 13.9 Hz, 2-CH_2), 2.84 (m, 1H, H-4), 2.81 (d, 3H, J = 4.9 Hz, CH_3-NH), 2.57 (m, 1H, H-3), 2.18 (m, 1H, H-3), 1.45 (d, 3H, J = 7.0 Hz, β-H, Ala). ^{1}H NMR (DMSO-d_6): cis/trans isomers ratio: 1:2.8. Trans isomer: δ 8.11 (d, 1H, J = 7.4 Hz, NH-Ala), 7.90 (m, 1H, NH-CH_3), 7.43−7.12 (m, 10H, Ph), 5.24 (d, 1H, J = 12.5 Hz, CH_2-Z), 5.03 (d, 1H, J = 12.5 Hz, CH_2-Z), 4.29 (m, 1H, α-H, Ala), 3.53 (m, 1H, H-4), 3.32 (d, 1H, J = 13.7 Hz, 2-CH_2), 2.99 (d, 1H, J = 13.7 Hz, 2-CH_2), 2.81 (m, 1H, H-4), 2.60 (d, 3H, J = 4.6 Hz, CH_3-NH), 2.33 (m, 1H, H-3), 2.06 (m, 1H, H-3), 1.25 (d, 3H, J = 7.1 Hz, β-H, Ala); cis isomer: δ 8.22 (d, 1H, J = 7.5 Hz, NH-Ala), 7.81 (m, 1H, NH-CH_3), 7.43−7.12 (m, 10H, Ph), 5.16 (m, 2H, CH_2-Z), 4.29 (m, 1H, α-H, Ala), 3.53 (m, 1H, H-4), 3.12 (m, 2H, 2-CH_2), 2.73 (m, 1H, H-4), 2.60 (d, 3H, J = 4.6 Hz, CH_3-NH), 2.19 (m, 1H, H-3), 2.06 (m, 1H, H-3), 1.15 (d, 3H, J = 7.1 Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): δ 174.3 and 172.4 (CON), 156.4 (OCON), 136.1, 134.7 (C-Ph), 130.4, 130.3, 128.6, 128.4, 127.1 (CH-Ph), 73.4 (2-C), 67.0 (CH_2-Z), 49.1 (α-C, Ala), 44.8 (4-C), 39.0 (2-CH_2), 26.3 (CH_3-NH), 23.5 (3-C), 17.3 (β-C, Ala).

ES-MS: 410.2 $[M + 1]^+$, 432.1 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{27}N_3O_4$: C 67.46, H 6.65, N 10.26, Found: C 67.25, H 6.72, N 10.10.

Ac-L-Pro-L-Ala-NHMe (12a):[43] Syrup, 11% yield (from **8a**, Method C). Eluent: MeOH:CH_2Cl_2 (1:10). $[\alpha]_D$ −60.71 (*c* 0.54, $CHCl_3$). ^1H NMR ($CDCl_3$): cis/trans isomers ratio: 1:6. Trans isomer: δ 7.09 (d, 1H, $J = 7.1$ Hz, NH-Ala), 6.53 (br s, 1H, NH-CH_3), 4.48 (m, 1H, α-H, Pro), 4.37 (q, 1H, $J = 7.1$ Hz, α-H, Ala), 3.60 (m, 1H, δ-H, Pro), 3.48 (m, 1H, δ-H, Pro), 2.78 (d, 3H, $J = 4.8$ Hz, CH_3-NH), 2.28 (m, 1H, β-H, Pro), 2.13 (s, 3H, CH_3-CO), 2.03 (m, 3H, β-H, Pro, γ-H, Pro), 1.36 (d, 3H, $J = 7.1$ Hz, β-H, Ala); cis isomer: δ 6.75 (d, 1H, $J = 7.1$ Hz, NH-Ala), 6.16 (br s, 1H, NH-CH_3), 4.48 (m, 1H, α-H, Pro), 4.37 (m, 1H, α-H, Ala), 3.60 (m, 1H, δ-H, Pro), 3.48 (m, 1H, δ-H, Pro), 2.78 (d, 3H, $J = 4.8$ Hz, CH_3-NH), 2.28 (m, 1H, β-H, Pro), 2.13 (s, 3H, CH_3-CO), 2.03 (m, 3H, β-H, Pro, γ-H, Pro), 1.36 (d, 3H, $J = 7.1$ Hz, β-H, Ala). ^1H NMR (DMSO-d_6): cis/trans isomers ratio: 1:2.4. Trans isomer: δ 7.88 (d, 1H, $J = 7.5$ Hz, NH-Ala), 7.56 (m, 1H, NH-CH_3), 4.22 (m, 1H, α-H, Pro), 4.15 (m, 1H, α-H, Ala), 3.48 (m, 2H, δ-H, Pro), 2.55 (d, 3H, $J = 4.6$ Hz, CH_3-NH), 2.03 (m, 2H, β-H, Pro), 1.98 (s, 3H, CH_3-CO), 1.83 (m, 2H, γ-H, Pro), 1.18 (d, 3H, $J = 7.5$ Hz, β-H, Ala); cis isomer: δ 8.16 (d, 1H, $J = 7.5$ Hz, NH-Ala), 7.82 (m, 1H, NH-CH_3), 4.35 (m, 1H, α-H, Pro), 4.28 (m, 1H, α-H, Ala), 3.48 (m, 2H, δ-H, Pro), 2.55 (d, 3H, $J = 4.6$ Hz, CH_3-NH), 2.15 (m, 2H, β-H, Pro), 1.98 (s, 3H, CH_3-CO), 1.83 (m, 2H, γ-H, Pro), 1.18 (d, 3H, $J = 7.5$ Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): trans isomer: δ 172.5 and 171.30 (CON), 171.33 (CO-CH_3), 60.2 (α-C, Pro), 49.1 (α-C, Ala), 48.5 (δ-C, Pro), 28.1 (β-C, Pro), 26.3 (CH_3-NH), 25.0 (γ-C, Pro), 22.6 (CH_3-CO), 17.6 (β-C, Ala); cis isomer: δ 172.5 and 171.30 (CON), 171.33 (CO-CH_3), 62.0 (α-C, Pro), 49.1 (α-C, Ala), 46.8 (δ-C, Pro), 29.7 (β-C, Pro), 26.3 (CH_3-NH), 25.0 (γ-C, Pro), 22.9 (CH_3-CO), 18.4 (β-C, Ala). ES-MS: 242.1 $[M + 1]^+$, 264.0 $[M + Na]^+$. Anal. Calcd for $C_{11}H_{19}N_3O_3$: C 54.76, H 7.94, N 17.41, Found: C 54.72, H 7.92, N 17.36.

Ac-L-α-MePro-L-Ala-NHMe (12b): Syrup, 32% yield (from **8b**, Method C). Eluent: MeOH:CH_2Cl_2 (1:15). $[\alpha]_D$ −48.30 (*c* 1.37, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.04 (br s, 1H, NH-CH_3), 6.44 (m, 1H, NH-Ala), 4.37 (q, 1H, $J = 7.5$ Hz, α-H, Ala), 3.65 (m, 2H, δ-H, Pro), 2.77 (d, 3H, $J = 4.6$ Hz, CH_3-NH), 2.28 (m, 1H, β-H, Pro), 2.13 (s, 3H, CH_3-CO), 2.09−1.88 (m, 3H, β-H, Pro, γ-H, Pro), 1.60 (s, 3H, α-CH_3, Pro), 1.39 (d, 3H, $J = 7.5$ Hz, β-H, Ala). ^1H NMR (DMSO-d_6): δ 7.76 (d, 1H, $J = 7.5$ Hz, NH-Ala), 7.50 (m, 1H, NH-CH_3), 4.16 (q, 1H, $J = 7.5$ Hz, α-H, Ala), 3.67 (m, 1H, δ-H, Pro), 3.57 (m, 1H, δ-H, Pro), 2.54 (d, 3H, $J = 4.5$ Hz, CH_3-NH), 1.99 (s, 3H, CH_3-CO), 1.97−1.78 (m, 4H, β-H, Pro, γ-H, Pro), 1.35 (s, 3H, α-CH_3, Pro), 1.22 (d, 3H, $J = 7.5$ Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): δ 173.4 and 172.8 (CON), 170.5 (CO-CH_3), 67.4 (α-C, Pro), 49.4 (δ-C, Pro), 49.2 (α-C, Ala), 39.1 (β-C, Pro), 26.2 (CH_3-NH), 23.8 (CH_3-CO), 23.5 (γ-C, Pro), 21.1 (α-CH_3, Pro), 17.7 (β-C, Ala). ES-MS: 256.0 $[M + 1]^+$, 278.0 $[M + Na]^+$, 533.3 $[2M + Na]^+$. Anal. Calcd for $C_{12}H_{21}N_3O_3$: C 56.45, H 8.29, N 16.46. Found: C 56.56, H 8.25, N 16.57.

Piv-L-Pro-L-Ala-NHMe (13a):[44] White solid (mp 85−87 °C), 55% yield (from **8a**, Method C). Eluent: MeOH:CH_2Cl_2 (1:20). $[\alpha]_D$ −56.45 (*c* 0.86, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.90 (m, 1H, NH-CH_3), 6.67 (d, 1H, $J = 7.3$ Hz, NH-Ala), 4.45 (m, 1H, α-H, Pro), 4.37 (q, 1H, $J = 7.3$ Hz, α-H, Ala), 3.71 (m, 2H, δ-H, Pro), 2.71 (d, 3H, $J = 4.7$ Hz, CH_3-NH), 2.07−1.87 (m, 4H, β-H, Pro, γ-H, Pro), 1.30 (d, 3H, $J = 7.3$ Hz, β-H, Ala), 1.24 (s, 9H, CH_3-^{t}Bu). ^1H NMR (DMSO-d_6): δ 7.72 (br s, 1H, NH-Ala), 7.64 (br s, 1H, NH-CH_3), 4.30 (m, 1H, α-H, Pro), 4.13 (q, 1H, $J = 7.2$ Hz, α-H, Ala), 3.63 (m, 2H, δ-H, Pro), 2.55 (d, 3H, $J = 4.6$ Hz, CH_3-NH), 2.07−1.87 (m, 4H, β-H, Pro, γ-H, Pro), 1.22 (br s, 3H, β-H, Ala), 1.16 (s, 9H, CH_3-^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 178.0 (CO-^{t}Bu), 172.6 and 172.0 (CON), 62.4 (α-C, Pro), 48.7 (α-C, Ala), 48.6 (δ-C, Pro), 39.0 (C-^{t}Bu), 27.6 (β-C, Pro), 27.4 (CH_3-^{t}Bu), 26.1 (CH_3-NH), 25.9 (γ-C, Pro), 17.9 (β-C, Ala). ES-MS: 284.0 $[M + 1]^+$, 306.0 $[M + Na]^+$, 589.3 $[2M + Na]^+$. Anal. Calcd for $C_{14}H_{25}N_3O_3$: C 59.34, H 8.89, N 14.83. Found: C 59.56, H 8.62, N 14.77.

Piv-L-α-MePro-L-Ala-NHMe (13b): Amorphous solid, 63% yield (from **8b**, Method C). Eluent: MeOH:CH_2Cl_2 (1:20). $[\alpha]_D$ −2.72 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.22 (br s, 1H, NH-CH_3), 5.71 (d, 1H, $J = 7.5$ Hz, NH-Ala), 4.45 (q, 1H, $J = 7.5$ Hz, α-H, Ala), 3.78 (m, 2H, δ-H, Pro), 2.75 (d, 3H, $J = 4.7$ Hz, CH_3-NH), 2.06−1.80 (m, 4H, β-H, Pro, γ-H, Pro), 1.56 (s, 3H, α-CH_3, Pro), 1.36 (d, 3H, $J = 7.5$ Hz, β-H, Ala) 1.26 (s, 9H, CH_3^{t}Bu). ^1H NMR (DMSO-d_6): δ 7.46 (m, 1H, NH-CH_3), 7.28 (m, 1H, $J = 7.3$ Hz, NH-Ala), 4.17 (q, 1H, $J = 7.3$ Hz, α-H, Ala), 3.86 (m, 1H, δ-H, Pro), 3.75 (m, 1H, δ-H, Pro), 2.54 (d, 3H, $J = 4.6$ Hz, CH_3-NH), 1.96 (m, 2H, γ-H, Pro), 1.86 (m, 1H, β-H, Pro), 1.72 (m, 1H, β-H, Pro), 1.32 (s, 3H, α-CH_3, Pro), 1.21 (d, 3H, $J = 7.3$ Hz, β-H, Ala), 1.18 (s, 9H, CH_3^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 177.4 (CO-^{t}Bu), 173.6 and 172.7 (CON), 69.0 (α-C, Pro), 49.0 (δ-C, Pro), 48.9 (α-C, Ala), 39.4 (C-^{t}Bu), 37.8 (β-C, Pro), 27.0 (CH_3-^{t}Bu), 26.5 (CH_3-NH), 24.8 (γ-C, Pro), 20.8 (α-CH_3, Pro), 17.9 (β-C, Ala). ES-MS: 298.0 $[M + 1]^+$, 320.0 $[M + Na]^+$, 617.3 $[2M + Na]^+$. Anal. Calcd for $C_{15}H_{27}N_3O_3$: C 60.58, H 9.15, N 14.13. Found: C 60.87, H 9.03, N 14.40.

2(*S*)-Ac-Aze-L-Ala-NHMe (14a): Solid (mp 152−154 °C), 64% yield (from **10a**, Method A). Eluent: MeOH:CH_2Cl_2 (1:10) (centrifugal circular thin-layer chromatography). $[\alpha]_D$ −197.35 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.91 (d, 1H, $J = 7.1$ Hz, NH-Ala), 6.79 (m, 1H, NH-CH_3), 4.79 (t, 1H, $J = 7.3$ Hz, H-2), 4.36 (q, 1H, $J = 7.1$ Hz, α-H, Ala), 4.04 (t, 2H, $J = 7.4$ Hz, H-4), 2.71 (d, 3H, $J = 4.6$ Hz, CH_3-NH), 2.50 (m, 1H, H-3), 2.42 (m, 1H, H-3), 1.87 (s, 3H, CH_3-CO), 1.31 (d, 3H, $J = 7.1$ Hz, β-H, Ala). ^1H NMR (DMSO-d_6): cis/trans isomers ratio: 1:1.3. Trans isomer: δ 8.13 (d, 1H, $J = 7.3$ Hz, NH-Ala), 7.74 (m, 1H, NH-CH_3), 4.60 (dd, 1H, $J = 9.0$, 5.9 Hz, H-2), 4.21 (q, 1H, $J = 7.3$ Hz, α-H, Ala), 4.02 (t, 2H, $J = 7.7$ Hz, H-4), 2.56 (m, 3H, CH_3-NH), 2.38 (m, 1H, H-3), 2.04 (m, 1H, H-3), 1.77 (s, 3H, CH_3-CO), 1.18 (d, 3H, $J = 7.3$ Hz, β-H, Ala); cis isomer: δ 8.28 (d, 1H, $J = 7.3$ Hz, NH-Ala), 7.86 (m, 1H, NH-CH_3), 4.80 (dd, 1H, $J = 9.0$, 5.1 Hz, H-2), 4.29 (q, 1H, $J = 7.3$ Hz, α-H, Ala), 3.73 (t, 2H, $J = 7.6$ Hz, H-4), 2.56 (m, 3H, CH_3-NH), 2.38 (m, 1H, H-3), 2.04 (m, 1H, H-3), 1.62 (s, 3H, CH_3-CO), 1.20 (d, 3H, $J = 7.3$ Hz, β-H, Ala). ^{13}C NMR ($CDCl_3$): trans isomer: δ 172.7 (CO-CH_3), 172.5 and 170.5 (CON), 61.6 (2-C), 49.0 (α-C, Ala), 48.5 (4-C), 26.1 (CH_3-NH), 18.8 (CH_3-CO), 18.4 (3-C), 17.6 (β-C, Ala); cis isomer: δ 172.7 (CO-CH_3), 172.5 and 171.0 (CON), 63.0 (2-C), 51.3 (α-C, Ala), 46.2 (4-C), 26.1 (CH_3-NH), 18.8 (CH_3-CO), 18.6 (3-C), 17.9 (β-C, Ala). ES-MS: 228.1 $[M + 1]^+$, 250.1 $[M + Na]^+$, 477.2 $[2M + Na]^+$. Anal. Calcd for $C_{10}H_{17}N_3O_3$: C 52.85, H 7.54, N 18.49. Found: C 52.94, H 7.61, N 18.37.

2(*S*)-Piv-Aze-L-Ala-NHMe (15a): White solid (mp 125−127 °C), 93% yield (from **11a**, Method A). Eluent: MeOH:CH_2Cl_2 (1:20). $[\alpha]_D$ −153.25 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.60 (m, 1H, NH-Ala), 6.80 (m, 1H, NH-CH_3), 4.78 (m, 1H, H-2), 4.36 (q, 1H, $J = 7.1$ Hz, α-H, Ala), 4.28 (t, 1H, $J = 7.8$ Hz, H-4), 2.81 (d, 3H, $J = 4.8$ Hz, CH_3-NH), 2.55 (m, 2H, H-3), 1.40 (d, 3H, $J = 7.1$ Hz, β-H, Ala), 1.24 (s, 9H, CH_3-^{t}Bu). ^1H NMR (DMSO-d_6): δ 7.99 (m, 1H, NH-Ala), 7.75 (m, 1H, NH-CH_3), 4.59 (m, 1H, H-2), 4.24 (m, 3H, α-H, Ala, H-4), 2.56 (d, 3H, $J = 4.6$ Hz, CH_3-NH), 2.38 (m, 1H, H-3), 2.05 (m, 1H, H-3), 1.18 (d, 3H, $J = 7.1$ Hz, β-H, Ala), 1.08 (s, 9H, CH_3-^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 179.8 (CO-^{t}Bu), 172.4 and 170.8 (CON), 62.4 (2-C), 51.8 (4-C), 48.5 (α-C, Ala), 38.6 (C-^{t}Bu), 29.5 (CH_3-NH), 26.9 (CH_3-^{t}Bu), 19.3 (3-C), 17.6 (β-C, Ala). ES-MS: 270.0 $[M + 1]^+$, 292.0 $[M + Na]^+$, 561.3 $[2M + Na]^+$. Anal. Calcd for $C_{13}H_{23}N_3O_3$: C 57.97, H 8.61, N 15.60. Found: C 58.04, H 8.91, N 15.87.

(43) Partial characterization details can be found in: Liang, G.-B.; Rito, C. J.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 4440−4442.

(44) Partial characterization details can be found in: (a) Aubry, A.; Cung, M. T.; Maraud, M. *J. Am. Chem. Soc.* **1985**, *107*, 7640−7647. (b) Boussard, G.; Maraud, M. *Biopolymers* **1979**, *18*, 1297−1331.

2(*S*)-Piv-MeAze-L-Ala-NHMe ((*S*)-15b): Syrup, 63% yield (from **9b**, Method C). Eluent: MeOH:CH_2Cl_2 (1:30). $[\alpha]_D$ −150.68 (*c* 0.52, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.18 (d, 1H, J = 7.3 Hz, NH-Ala), 6.55 (br s, 1H, NH-CH_3), 4.40 (q, 1H, J = 7.3 Hz, α-H, Ala), 4.28 (m, 2H, H-4), 2.77 (d, 3H, J = 4.9 Hz, CH_3-NH), 2.07 (m, 2H, H-3), 1.74 (s, 3H, 2-CH_3), 1.37 (d, 3H, J = 7.3 Hz, β-H, Ala), 1.20 (s, 9H, CH_3-^{t}Bu). ^{1}H NMR (DMSO-d_6): δ 8.07 (d, 1H, J = 7.2 Hz, NH-Ala), 7.71 (m, 1H, NH-CH_3), 4.29 (m, 2H, H-4), 4.21 (q, 1H, J = 7.2 Hz, α-H, Ala), 2.57 (d, 3H, J = 4.6 Hz, CH_3-NH), 2.40 (m, 1H, H-3), 1.90 (m, 1H, H-3), 1.55 (s, 3H, 2-CH_3), 1.21 (d, 3H, J = 7.2 Hz, β-H, Ala), 1.12 (s, 9H, CH_3-^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 179.6 (CO-^{t}Bu), 174.1 and 172.6 (CON), 72.0 (2-C), 49.4 (4-C), 49.1 (α-C, Ala), 38.9 (C-^{t}Bu), 27.7 (3-C), 26.7 (CH_3-^{t}Bu), 26.3 (CH_3-NH), 22.5 (2-CH_3), 17.4 (β-C, Ala). ES-MS: 284.0 $[M + 1]^+$, 306.0 $[M + Na]^+$, 589.3 $[2M + Na]^+$. Anal. Calcd for $C_{14}H_{25}N_3O_3$: C 59.34, H 8.89, N 14.83. Found: C 59.17, H 8.91, N 14.71.

2(*R,S*)-Piv-MeAze-L-Ala-NHMe ((*R,S*)-15b): Syrup, 88% yield (from (*R,S*)-**4b**, Method B). Eluent: MeOH:CH_2Cl_2 (1:30). ^{1}H NMR ($CDCl_3$): diastereoisomers ratio: 1.2:1. Major diastereoisomer: (*S*)-**15b** (described above); minor diastereoisomer: δ 7.88 (d, 1H, J = 7.3 Hz, NH-Ala), 6.72 (br s, 1H, NH-CH_3), 4.40 (q, 1H, J = 7.3 Hz, α-H, Ala), 4.30 (m, 2H, H-4), 2.77 (d, 3H, J = 4.9 Hz, CH_3-NH), 2.04 (m, 2H, H-3), 1.74 (s, 3H, 2-CH_3), 1.40 (d, 3H, J = 7.3 Hz, β-H, Ala), 1.19 (s, 9H, CH_3-^{t}Bu). ^{1}H NMR (DMSO-d_6): Major diastereoisomer: (*S*)-**15b** (described above); minor diastereoisomer: δ 7.95 (d, 1H, J = 7.2 Hz, NH-Ala), 7.82 (m, 1H, NH-CH_3), 4.29 (m, 2H, H-4), 4.21 (q, 1H, J = 7.2 Hz, α-H, Ala), 2.57 (d, 3H, J = 4.6 Hz, CH_3-NH), 2.40 (m, 1H, H-3), 1.90 (m, 1H, H-3), 1.60 (s, 3H, 2-CH_3), 1.21 (d, 3H, J = 7.2 Hz, β-H, Ala), 1.12 (s, 9H, CH_3-^{t}Bu). ^{13}C NMR ($CDCl_3$): major diastereoisomer: (*S*)-**15b** (described above); minor diastereoisomer: δ 179.5 (CO-^{t}Bu), 173.6 and 172.6 (CON), 71.4 (2-C), 49.8 (4-C), 49.3 (α-C, Ala), 38.8 (C-^{t}Bu), 27.4 (3-C), 26.7 (CH_3-^{t}Bu), 26.3 (CH_3-NH), 22.4 (2-CH_3), 17.3 (β-C, Ala). ES-MS: 284.0 $[M + 1]^+$, 306.0 $[M + Na]^+$, 589.3 $[2M + Na]^+$. Anal. Calcd for $C_{14}H_{25}N_3O_3$: C 59.34, H 8.89, N 14.83. Found: C 59.03, H 9.01, N 14.59.

2(*S*)-Piv-BnAze-L-Ala-NHMe (15c): Syrup, 90% yield (from **9a**, Method C). Eluent: MeOH:CH_2Cl_2 (1:30). HPLC: t_R = 3.77 min (A:B = 40:60). $[\alpha]_D$ −5.17 (*c* 0.97, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.55 (d, 1H, J = 7.2 Hz, NH-Ala), 7.35−7.20 (m, 5H, Ph), 6.49 (br s, 1H, NH-CH_3), 4.41 (q, 1H, J = 7.2 Hz, α-H, Ala), 3.95 (ddd, 1H, J = 9.8, 8.3, 5.3 Hz, H-4), 3.68 (d, 1H, J = 13.9 Hz, 2-CH_2), 3.38 (m, 1H, H-4), 3.04 (d, 1H, J = 13.9 Hz, 2-CH_2), 2.77 (d, 3H, J = 4.9 Hz, CH_3-NH), 2.66 (ddd, 1H, J = 12.1, 9.8, 7.2 Hz, H-3), 2.26 (ddd, 1H, J = 12.1, 9.4, 5.3 Hz, H-3), 1.39 (d, 3H, J = 7.2 Hz, β-H, Ala), 1.13 (s, 9H, CH_3-^{t}Bu). ^{1}H NMR (DMSO-d_6): δ 8.35 (d, 1H, J = 7.2 Hz, NH-Ala), 7.78 (m, 1H, NH-CH_3), 7.34−7.20 (m, 5H, Ph), 4.27 (q, 1H, J = 7.2 Hz, α-H, Ala), 4.04 (m, 1H, H-4), 3.46 (d, 1H, J = 13.3 Hz, 2-CH_2), 3.24 (m, 1H, H-4), 2.97 (d, 1H, J = 13.3 Hz, 2-CH_2), 2.60 (d, 3H, J = 4.7 Hz, CH_3-NH), 2.34 (m, 1H, H-3), 2.10 (m, 1H, H-3), 1.23 (d, 3H, J = 7.2 Hz, β-H, Ala), 1.06 (s, 9H, CH_3-^{t}Bu). ^{13}C NMR ($CDCl_3$): δ 179.7 (CO-^{t}Bu), 173.9 and 172.5 (CON), 135.1 (C-Ph), 130.6, 130.0, 128.3 (CH-Ph), 75.5 (2-C), 49.6 (4-C), 49.1 (α-C, Ala), 39.1 (C-^{t}Bu), 38.7 (2-CH_2), 26.9 (CH_3-^{t}Bu), 26.3 (CH_3-NH), 23.5 (3-C), 17.2 (β-C, Ala). ES-MS: 382.0 $[M + Na]^+$. Anal. Calcd for $C_{20}H_{29}N_3O_3$: C 66.83, H 8.13, N 11.69. Found: C 66.76, H 8.01, N 11.59.

Molecular Modeling Studies. All calculations were run on an SGI workstation (Fuel, RP14000, 500 MB RAM) under an Irix 6.5 operating system. The initial conformation was built using the library of fragments available in the molecular modeling program Insight II (version 2000.1, Biosym Technologies, San Diego, CA). The calculations were carried out within the molecular mechanics using the AMBER force field implemented in DISCOVER. They were conducted under vacuum with a distance dependent dielectric constant (4*r*) and a cutoff of 16 Å. The structure is heated to 1000 K and equilibrated during 100 ps. The structure is then cooled slowly to 300 K in steps; in each step the temperature was lowered by 100 deg, and the system was allowed to stay at the new temperature for 100 ps. After cooling to 300 K, the final conformation obtained is energy-refined using a conjugated gradient algorithm with a final gradient of 0.001 kcal/mol as the convergence criteria. The conformer is stored and used to start a new simulation at high temperature. This procedure produced samples of 100 energy-minimized conformations, which were compared to each other to eliminate the identical ones. The protocol was run three times, employing different starting structures. Independently of the starting structure, the percentage of the different families of conformers was equivalent.

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Supporting Information Available: Experimental procedures and analytical and spectroscopic data of compounds **1**, **3**−**4**, **28**, **16**−**23**, **25**, and **26**, relevant conformational parameters of dipeptide derivatives **12a**, **12b**, **14a**, and **14b**, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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