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Azepane Quaternary Amino Acids As Effective Inducers of 3_{10} Helix Conformations

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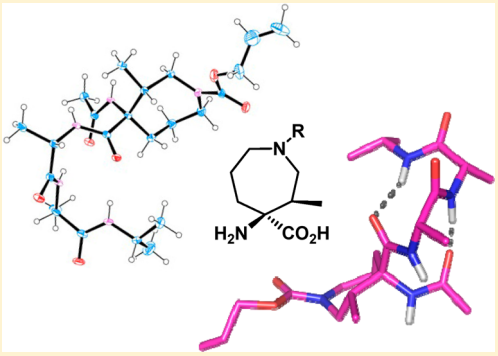
Supporting Information

ABSTRACT: A simple method for the synthesis of an azepane quaternary amino acid in enantiopure form is described. Theoretical, NMR, and X-ray studies indicated that this azepane-derived amino acid is an effective stabilizer of 3_{10} helical structures in short peptides.



The relevant role of secondary peptide structure in peptide−protein or protein−protein interactions has prompted the research for scaffolds able to induce or mimic these motifs.[1−3] In this sense, C^{α}-tetrasubstituted amino acids are useful tools to reduce the conformational space available to peptides, especially when the α,α substituents are incorporated within a ring structure. Studies on peptides incorporating cyclic quaternary α-amino acids indicated their general preference for adopting turn or helical structures.[4,5] However, quite frequently, these studies have been carried out with peptides that also contain another restricted amino acid, as Pro or Aib, that by themselves have a marked tendency toward particular secondary structure elements.[6,7] Only a few reports deal with conformationally constrained peptides in which the restriction is exclusively imposed by cyclic quaternary amino acids. For instance, the seven member cycloaliphatic derivative, 1-aminocycloheptane-1-carboxylic acid, led to the adoption of β-turns in tetrapeptide models (Figure 1, I).[8] Related to heterocyclic tetrasubstituted amino acids, the achiral 1,2-dithiolane derivative (**II**) was also able to induce β-turns,[9] while the chiral tetrahydrofuran-derived amino acid *RSS*-TAA (**III**), prepared as racemic, stabilized either β-turns or distorted 3_{10} helix structures.[10] The achiral 4-piperidine, and specially its *N*-oxide analogue TOAC (**IV**), are by far the most widely studied heterocyclic quaternary amino acids, which have been described as effective β-turn and $3_{10}/\alpha$-helix inducers in peptides, especially in combination with Aib residues.[6,11,12] In absence of any other structuring element, the incorporation of two TOAC residues in medium-size peptides favors the adoption of either 3_{10} or α-helical structures,[13] while just one residue induces a right-handed helix, starting by a 3_{10} helix and changes to a two consecutive α-turn motif near the C-terminus.[14]

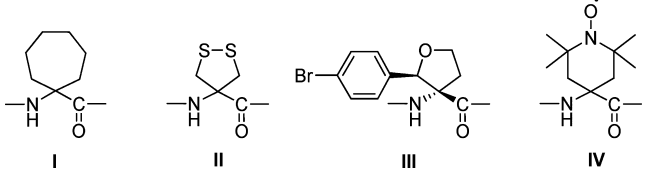


Figure 1. Structure of some cyclic and heterocyclic tetrasubstituted amino acids.

Last year, we have described quaternary α,α-2-oxoazepane α-amino acid derivatives, like **1**, that when incorporated into the i + 1 position of tetrapeptide models are able to induce β-turn secondary structures.[15] Compound **1** can be synthesized from commertially available H-Orn(Z)-OMe in seven steps with an overall yield of 32%.[15] In these models, the 2-CO group of the heterocyclic ring participates in an intramolecular H-bond with the oxoazepane 4-NH proton, thus contributing to the global 3D arrangement. However, this intraresidue H-bond could be a handicap when the α,α-quaternary amino acid occupies other positions within the peptide, since it could hamper the participation of this NH proton in hydrogen bonds with other residues, like in helix conformations. To circumvent this issue, here we describe the selective transformation of compound **1** to the orthogonally protected azepane amino acid derivative **3**, and its influence on the adoption of stable

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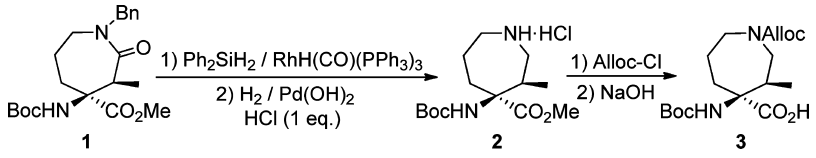
Scheme 1. Synthesis of the Azepane-Derived Quaternary Amino Acid 3

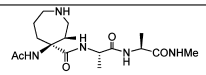
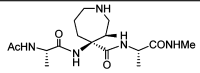
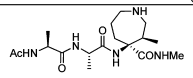


peptide secondary structures when incorporated into pentapeptide models. In addition, compound **3** could be suitable for further derivatization to allow side-chain cross-linking in peptides, as previously described for the lower piperidine homologue.[16]

The convenient synthetic method implied the selective reduction of lactam **1** with diphenylsilane in the presence of a rhodium catalyst,[17] followed by catalytic hydrogenolysis to remove the benzyl group (Scheme 1). Next, treatment of the resulting azepane **2** with Alloc-chloride and the subsequent hydrolysis of the ester gave compound **3**, suitably protected for the incorporation into peptide sequences (64% overall yield from **1**). All the steps proceeded with complete preservation of the configurational integrity of the stereocenters, leading to enantiopure **3**.

To characterize the conformational preferences of small peptides that incorporate our azepane derived amino acid, we decided to study their ability to induce turns or helical structures. To this aim, we selected model systems able to mimic pentapeptides, Ac-Xaa-Yaa-Zaa-NHMe[18] in which two of the residues are Ala, and the third one is our template (Table 1, **A**–**C**). In these models, any bias toward a particular 3D

Table 1. Theoretical Percentage of Conformers with Reverse Turns

	% reverse turns[a]		
Peptide derivative	β^{i-i+3}	$\beta^{i+1-i+4}$	α^{i-i+4}
 Ac-Aze-Ala-Ala-NHMe (**A**)	100 %	49 %	51 %
 Ac-Ala-Aze-Ala-NHMe (**B**)	97 %	92 %	8 %
 Ac-Ala-Ala-Aze-NHMe (**C**)	72 %	93 %	0 %

[a]Within a 3 kcal/mol window from the global minimum.

structure could be solely attributed to the azepane derivative contribution. Theoretical studies, carried out by molecular dynamic simulations using Amber as the force field, indicated that the azepane containing peptides **A**–**C** have a marked tendency to adopt either α or β-turns (Table 1). Analysis of the dihedral angles of the minimum energy families showed that either the global minimum in **B** and **C**, or a family over 0.2 kcal/mol from the global minimum in **A**, is able to adopt two consecutive type III β-turns, therefore constituting two turns of a 3_{10} helix,[19] in which the characteristic hydrogen bonds are observed (Figure 2, and Supporting Information Table S1). Additionally, the global minimum of peptide **A** and the second family of low energy in **B** are a combination of a type I-α_{RS} and type I or III β-turns.

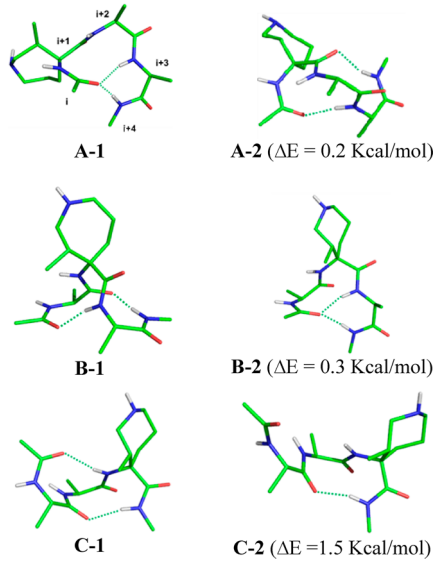


Figure 2. Representative minimal energy conformers of tripeptides that incorporate the azepane amino acid at i +1 (A), i + 2 (B) and i + 3 (C).

On the whole, our molecular modeling studies suggest that the azepane residue is an efficient inducer of reverse turns, with higher structure degree when the restricted amino acid moves toward the *N*-terminal part of the model peptides (**A** and **B**).

To get additional knowledge about the conformation induced by the azepane-derived amino acid into peptides, we selected a few pentapeptide models to be synthesized and further studied. Thus, tripeptide derivatives **4** and **5** were obtained from amino acid **3** by coupling with the corresponding H-Ala-Ala-NHR[3] dipeptide amides (Scheme 2). Removal of the Alloc group from compound **5** yielded azepane-deprotected analogue **6**, while the *N*-Boc deprotection, followed by acetylation, gave the Ac-tripeptide derivative **7**. In a

Scheme 2. Synthesis of Azepane-Containing Tripeptide Derivatives

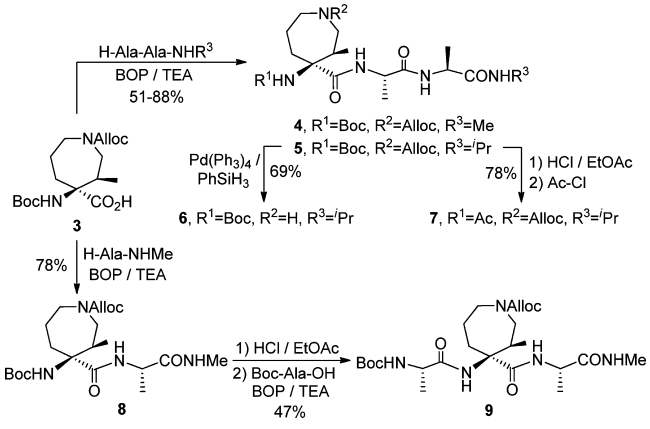


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Table 2. Chemical Shifts of NH Protons in $CDCl_3$ and DMSO-d_6

	δ ($CDCl_3$)[a]				δ (DMSO)[a,b]			
compd	NH^{i+1}	NH^{i+2}	NH^{i+3}	NH^{i+4}	NH^{i+1}	NH^{i+2}	NH^{i+3}	NH^{i+4}
4	4.82, 5.00	6.48	7.57	6.89	6.95, 7.02 (1.95−2.20)	8.00 (1.52)	7.85 (0.28)	7.41, 7.48 (0.52−0.59)
5	5.03, 5.13	6.50	7.59	6.53, 6.57	7.01, 7.07 (1.98−2.04)	8.09 (1.59)	7.83 (0.24)	7.15, 7.18 (0.58−0.65)
6[c]					7.30	8.23	7.84	7.34
7	6.45	6.53, 6.70	7.56, 7.63	6.67, 6.73	7.80, 7.82 (1.35−1.37)	8.13 (1.43−1.60)	7.73 (0.10−0.17)	7.07, 7.10 (0.34−0.43)
9	5.32	6.55, 6.57	7.45, 7.47	7.16	7.31, 7.32 (1.99−2.00)	7.46, 7.48 (0.93−0.89)	7.58, 7.59 (0.11−0.14)	7.46 (0.30)

[a]NH were assigned on the basis of COSY and ROESY experiments; see the Supporting Information. When there are two NH signals, each corresponds to one of the rotamers. [b]$\Delta\delta_{DMSO-CDCl3}$ values are indicated in parentheses. [c]Not soluble in $CDCl_3$.

similar way, the incorporation of the constrained amino acid at the $i + 2$ position of the model peptide was carried out by sequential deprotection and coupling, first at the *C*-terminal and then at the *N*-terminal part, affording the corresponding tripeptide derivative **9**.

The conformational preferences of tripeptides **4**−**7** and **9** were analyzed by ^{1}H NMR; in particular, we studied the chemical shift of amide protons in $CDCl_3$ and their variation when the solvent was changed to DMSO-d_6 (Table 2). As well, we evaluated the temperature coefficient values ($\Delta\delta/\Delta T$, Table 3). As expected, the presence of an Alloc substituent on the heterocyclic NH give rise to two sets of signals corresponding to the rotamers of the urethane moiety, which coalesce when increasing the temperature. For the conformational studies, whenever possible both sets of signals were considered.

Table 3. Temperature Coefficients for NH Protons

	$\Delta\delta/\Delta T$ (ppb/K)[a,b]			
compd	NH^{i+1}	NH^{i+2}	NH^{i+3}	NH^{i+4}
4	−7.6, −9.6	−8.6	**−2.7**	**−0.4, −1.6**
5	−7.6, −8.2	−7.7	**−2.6**	**−0.2, −2.1**
6	−6.6	−6.2	**−2.5**	**−1.4**
7	−4.3, −4.3	−6.0	**−2.0**	**−1.6, −1.5**
9	−4.7	−4.3	−3.7	**−2.0**

[a]NH were assigned on the basis of COSY and ROESY experiments. When there are two NH coefficients, each corresponds to one of the rotamers. [b]$\Delta\delta$ measured in DMSO-d_6, 30−45 °C (each 5 °C for a total of 4 points), except for compound **9** measured in DMSO-d_6, 45−60 °C (each 5 °C for a total of 4 points); samples at 7−10 mM concentration.

Values of δ ($CDCl_3$) higher than 7 ppm and small variation of $\Delta\delta_{DMSO-CDCl_3}$ are indicative of NHs shielded from solvent.[20] Taking into consideration this criteria, NH^{i+3} is solvent-shielded, whereas the others NH have a chemical shift in $CDCl_3$ below 7 ppm. However, the chemical shift of NH^{i+4} is close to 7.00 ppm, and its variation when the solvent is changed to DMSO-d_6 is small (0.3−0.6 ppm), which might be suggestive of this proton also being involved in a hydrogen bond. On the other hand, $\Delta\delta/\Delta T$ coefficients below 3 ppb·K^{-1} (in absolute value) are indicative of solvent-shielded NH protons, which in small peptides suggest their participation in intramolecular hydrogen bonds, whereas values above 4 ppb·K^{-1} indicate NH protons accessible to solvent.[20] If we consider variable temperature studies, when the restricted amino acid is incorporated at the $i + 1$ position, as in peptide derivatives **4**−**7**, the temperature coefficient values for NH^{i+3} and NH^{i+4} amide protons are pinpointing to their involvement in hydrogen bonds. On the contrary, the variation of the chemical shift when the solvent is changed from $CDCl_3$ to DMSO-d_6 for NH^{i+1} and NH^{i+2} is between 0.9 and 2.2 pm, which is indicative of solvent accessible NHs. It is interesting to mention that different acyl groups at *N*-terminus (**4** vs **7**) or variable alkyl amides at *C*-terminal (**4** vs **5**) were always compatible with the existence of these hydrogen bonds. In a similar manner, the heterocyclic amino group can be either protected or deprotected without significant influence on the ability of the peptides to be structured (**5** vs **6**). When the quaternary amino acid is at the $i + 2$ position, as in compound **9**, the $i + 4$ amide proton is also solvent protected, whereas the temperature coefficient for the NH^{i+3} is in the uncertainty range, suggesting a slightly lower stabilization of folded structures. However, the small chemical shift variation of the NH^{i+3} proton in **9** when the solvent is changed from $CDCl_3$ to DMSO-d_6 indicated once more that both amide protons (NH^{i+3} and NH^{i+4}) are involved in H-bonds. The experimental temperature coefficients measured for peptides **4**−**7** and **9** can be correlated with the two main secondary structures predicted by the theoretical studies, either a 3_{10} helix or a combination of an α- and a β-turn. Unfortunately, no conclusive data toward any of these conformations could be obtained by examining the NOESY and ROESY 2D-spectra.

Further insights into the conformational preference of these derivatives were obtained from the crystal structure of compound **7**.[21] Tripeptide derivative **7** crystallizes with an EtOAc molecule that participates in stabilizing the crystal packing (see Supporting Information for details). In the solid state, peptide **7** assumes a 3_{10} helix conformation, having the characteristic hydrogen bonds of this structure. The first H-bond is formed between the CO group of the acetyl moiety and the Ala NH^{i+3} amide proton (distance N···O 2.9 Å and angle N—H···O 160.8°), while the second is formed between the carbonyl of the azepane residue and the *C*-terminal NH^{i+4} proton (distance N···O 3.1 Å and angle N—H···O 158.7°). Additionally, the dihedral angles match very well to those expected for this type of helical structure ($\phi_{i+1} = -59.9$; $\psi_{i+1} = -27.3$; $\phi_{i+2} = -61.5$; $\psi_{i+2} = -21.9$; $\phi_{i+3} = -78.3$; $\psi_{i+3} = -18.0$) (Figure 3). A good overall superposition is observed between the X-ray structure and one of the minimum energy families, **A2**.

In conclusion, we have presented a facile synthesis of an orthogonally protected azepane-containing α,α-tetrasubstituted amino acid derivative (3*R*,4*S*-configuration). We have also shown that when incorporated into model pentapeptides, this quaternary amino acid is able to efficiently induce the adoption

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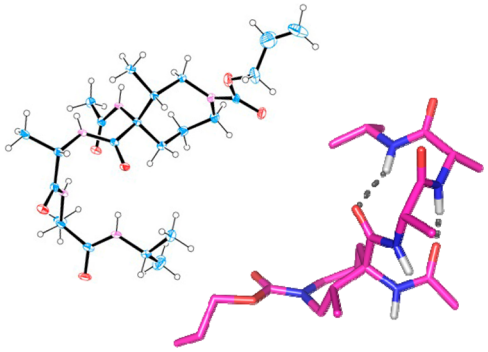

Figure 3. X-ray structure of derivative 7 [Ac-Aze(Alloc)-Ala-Ala-NH^{i}Pr]. In the ORTEP view, ellipsoids are drawn at 30% probability level.

of 3_{10} helical structures, both in solution and in solid state, thus constituting a new tool to stabilize the conformational preferences of short peptides.[22] Since the incorporation of substituents at the NH group of the azepane ring seems a priori straightforward, the preparation of diverse peptide derivatives for use as biological probes could be easily envisaged.

EXPERIMENTAL SECTION

Molecular Modeling Studies. Molecular dynamic simulations were carried out using the Amber 10 suite of programs with the ff99SB force field. Antechamber was used to assign atom types to model tripeptides and to calculate a set of point charges using the AM1-BCC charge model. The Hawkins, Cramer, Truhlar pairwise generalized Born model was used to simulate implicit waters. The molecules were relaxed by energy minimization, and then a molecular dynamic simulation was carried out during 40 ps at constant temperature (300 K). Then the system was heated to 1000 K during 350 ps and allowed to stay at this temperature for 100 ps. The structures were subsequently cooled slowly to 300 K in steps; in each step the temperature was lowered by 100 K, and the system was allowed to stay at the new temperature for 100 ps, with 200 ps extra at 300 K. The final conformation obtained was energy-refined using steepest descent followed by conjugate gradient algorithm with a final gradient of 0.001 kcal/mol as the convergence criteria. The conformers were stored and used to start a new simulation at high temperature. This procedure afforded samples of 100 energy-minimized conformations, which were compared to each other to eliminate the identical ones. The resulting structures were visually inspected using the computer program VMD and analyzed using the *ptraj* analysis program within Amber.

(3*R*,4*S*)-4-(*tert*-Butoxycarbonylamino)-4-methoxycarbonyl-3-methylazepane hydrochloride (2). A solution of compound **1** (0.137 g, 0.35 mmol) in dry THF (8 mL) under Ar atmosphere was treated with $RhH(CO)(PPh_3)_3$ (0.010 g, 0.01 mmol) and Ph_2SiH_2 (0.163 mL, 0.88 mmol), and stirred at room temperature for 40 h. After evaporation of the solvent, the residue was purified on a silica gel column, using $MeOH/CH_2Cl_2$ (1:90), yielding (3*R*,4*S*)-1-Benzyl-4-(*tert*-butoxycarbonyl)amino-4-methoxycarbonyl-3-methylazepane as a syrup: yield, 0.119 g, 90%; $[\alpha]_D^{20}$ = +14.8 (*c* 0.9, $CHCl_3$); HPLC t_R = 3.43 min (protonated amine) and 3.54 min (deprotonated amine) (gradient A/B from 10:90 to 100:0 over 5 min); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.72 (d, 3H, *J* = 7.1, 3-CH_3), 1.39 (s, 9H, CH_3, Boc), 1.56 (m, 2H, 6-H), 2.06 (m, 1H, 5-H), 2.21 (m, 1H, 3-H), 2.43 (m, 1H, 5-H), 2.54 (m, 4H, 7-H, 2-H), 3.50 (d, 1H, *J* = 13.4, 1-CH_2), 3.57 (d, 1H, *J* = 13.4, 1-CH_2), 3.64 (s, 3H, OCH_3), 5.21 (s, 1H, 4-NH), 7.15–7.30 (m, 5H, CH, Bn); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.8 (3-CH_3), 24.1 (6-C), 28.5 (CH_3, Boc), 33.4 (5-C), 40.4 (3-C), 52.2 (OCH_3), 55.4 (7-C), 59.1 (2-C), 63.6 (1-CH_2), 64.6 (4-C), 79.5 (C, Boc), 127.2, 128.5, 128.9, and 139.7 (1-C and CH, Bn), 155.7 (CO, Boc), 174.6 (COO); MS (ESI) *m*/*z* = 377.5 $[M + H]^+$, 378.5 $[M + 2H]^+$. Elemental analysis calcd (%) for $C_{21}H_{32}N_2O_4$: C 66.99, H 8.57, N 7.44. Found (%): C 66.81, H 8.62, N 7.30.

A solution of this *N*-benzyl azepane derivative (0.070 g, 0.19 mmol) in MeOH (10 mL) was treated with 37% HCl (0.016 mL, 0.19 mmol) and $Pd(OH)_2$ (0.021 g, 30% w/w). The suspension was hydrogenated at 50 °C and 45 psi for 12 h. After filtration of the catalyst, the solvent was evaporated. Product **2** was used for the following reaction without further purification. Syrup: 0.061 g, quantitative yield; $[\alpha]_D^{20}$ = +5.5 (*c* 0.9, $CHCl_3$); HPLC t_R = 1.24 min (gradient A/B from 2:98 to 5:95 over 20 min); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (d, 3H, *J* = 7.1, 3-CH_3), 1.36 (s, 9H, CH_3, Boc), 1.68 (m, 2H, 6-H), 2.16 (m, 1H, 5-H), 2.27 (m, 1H, 5-H), 2.54 (m, 1H, 3-H), 2.92 (m, 2H, 2-H, 7-H), 3.12 (m, 2H, 2-H, 7-H), 3.60 (s, 3H, OCH_3), 7.06 (s, 1H, 4-NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.6 (3-CH_3), 19.0 (6-C), 28.1 (CH_3, Boc), 32.3 (5-C), 37.3 (3-C), 45.0 (7-C), 46.5 (2-C), 52.0 (OCH_3), 63.3 (4-C), 78.4 (C, Boc), 155.0 (CO, Boc), 173.5 (COO); MS (ESI) *m*/*z* = 287.3 $[M - HCl + H]^+$. Elemental analysis calcd (%) for $C_{14}H_{27}N_2O_4Cl$: C 52.09, H 8.43, N 8.68. Found (%): C 52.04, H 8.61, N 8.30.

(3*R*,4*S*)-1-Allyloxycarbonyl-4-(*tert*-butoxycarbonyl)amino-4-carboxy-3-methylazepane (3). Compound **2** (0.060 g, 0.19 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and TEA (0.026 mL, 0.19 mmol), propylene oxide (0.195 mL, 2.79 mmol) and Alloc-Cl (0.040 mL, 0.37 mmol) were successively added. The solution was stirred at room temperature for 12 h. After evaporation of the solvent, the residue was purified on a silica gel column, using EtOAc/hexane (1:3), leading to (3*R*,4*S*)-1-allyloxycarbonyl-4-(*tert*-butoxycarbonylamino)-4-methoxycarbonyl-3-methylazepane as a syrup: yield, 0.052 g, 76%; $[\alpha]_D^{20}$ = +12.8 (*c* 1.0, $CHCl_3$); HPLC t_R = 14.68 min (major rotamer) and 15.92 (minor rotamer) (gradient A/B from 5:95 to 80:20 over 20 min); ^{1}H NMR (300 MHz, $CDCl_3$, two rotamers, Mr/mr = 1.2:1) δ 0.86 (d, 3H, *J* = 7.1, 3-CH_3, mr), 0.87 (d, 3H, *J* = 7.1, 3-CH_3, Mr), 1.44 (s, 9H, CH_3, Boc), 1.76 (m, 2H, 6-H), 1.88 (m, 1H, 5-H), 2.33 (m, 1H, 3-H), 2.61 (m, 1H, 5-H), 3.02 (m, 1H, 2-H), 3.21 (m, 1H, 7-H), 3.63 (m, 2H, 2-H, 7-H), 3.71 (s, 3H, OCH_3, Mr), 3.72 (s, 3H, OCH_3, mr), 4.56 (s, 1H, 4-NH), 4.59 (m, 2H, 1′-H, Alloc), 5.21 (dq, 1H, *J* = 10.6 and 1.4, 3′-H, Alloc), 5.30 (ddt, 1H, *J* = 17.2, 2.4 and 1.4, 3′-H, Alloc), 5.94 (ddt, 1H, *J* = 17.2, 10.6 and 5.4, 2′-H, Alloc); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9 (3-CH_3), 21.3 (6-C, mr), 21.9 (6-C, Mr), 28.5 (CH_3, Boc), 31.8 (5-C), 41.2 (3-C, Mr), 41.5 (3-C, mr), 46.3 (7-C, Mr), 46.5 (7-C, mr), 47.7 (2-C, Mr), 48.5 (2-C, mr), 52.5 (OCH_3, Mr), 52.6 (OCH_3, mr), 64.4 (4-C, mr), 64.5 (4-C, Mr), 66.2 (1′-C, Alloc), 80.1 (C, Boc, mr), 80.2 (C, Boc, Mr), 117.4 (3′-C, Alloc, mr),117.5 (3′-C, Alloc, Mr), 133.3 (2′-C, Alloc), 155.5 and 156.1 (CO, Alloc and Boc, mr), 155.7 and 156.5 (CO, Alloc and Boc, Mr), 174.4 (COO, Mr), 174.5 (COO, mr); MS (ESI) *m*/*z* = 393.2 $[M + Na]^+$. Elemental analysis calcd (%) for $C_{18}H_{30}N_2O_6$: C 58.36, H 8.16, N 7.56. Found (%): C 58.11, H 8.17, N 7.49.

A solution of that resulting 1-allyloxycarbonyl-azepane derivative (0.052 g, 0.14 mmol) in MeOH (2 mL) was treated with 2N NaOH (0.702 mL, 1.40 mmol) and stirred at 65 °C for 48 h. Then, the solvent was evaporated to dryness, and the residue was dissolved in H_2O and washed with EtOAc. The aqueous phase was acidified with 1 M HCl to pH 3 and extracted with EtOAc. The organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. Product **3** was used for the following reaction without further purification. Solid: mp 83–86 °C (EtOAc); yield, 0.046 g, 93%; $[\alpha]_D^{20}$ = +14.5 (*c* 0.7, $CHCl_3$); HPLC t_R = 4.94 min (gradient A/B from 10:90 to 100:0 over 5 min); ^{1}H-RMN (300 MHz, $CDCl_3$, two rotamers, Mr/mr = 1.1:1) δ 0.93 (d, 3H, *J* = 6.9, 3-CH_3, mr), 0.94 (d, 3H, *J* = 7.0, 3-CH_3, Mr), 1.44 (s, 9H, CH_3, Boc), 1.73 (m, 1H, 5-H), 1.86 (m, 2H, 6-H), 2.38 (m, 1H, 3-H), 2.63 (m, 1H, 5-H), 3.04 (m, 1H, 2-H), 3.18 (m, 1H, 7-H), 3.70 (m, 2H, 2-H, 7-H), 4.60 (m, 2H, 1′-H, Alloc), 4.65 (s, 1H, 4-NH), 5.21 (dq, 1H, *J* = 10.6 and 1.5, 3′-H, Alloc), 5.29 (dq, 1H, *J* = 17.1 and 1.5, 3′-H, Alloc), 5.92 (ddt, 1H, *J* = 17.1, 10.6 and 5.4, 2′-H, Alloc); ^{13}C-RMN (75 MHz, $CDCl_3$) δ 13.9 (3-CH_3), 21.2 (6-C, mr), 21.9 (6-C, Mr), 28.5 (CH_3, Boc), 31.5 (5-C, mr), 31.7 (5-C, Mr), 41.1 (3-C, Mr), 41.3 (3-C, mr), 46.3 (7-C, mr), 46.4 (7-C, Mr), 47.6 (2-C, mr), 48.4 (2-C, Mr), 64.3 (4-C), 66.2 (1′-C, Alloc), 80.7 (C, Boc), 117.5 (3′-C, Alloc), 133.2 (2′-C, Alloc), 156.2 and 156.5 (CO, Alloc and Boc), 177.7 (COO, Mr), 177.8 (COO, mr); MS (ESI) *m*/*z* =

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379.2 [M + Na]$^+$. Elemental analysis calcd (%) for $C_{17}H_{28}N_2O_6$: C 57.29, H 7.92, N 7.86. Found (%): C 57.00, H 8.23, N 7.81.

General Procedure for the Synthesis of Boc-Aze(Alloc)-Ala-Ala-NHR Tripeptides. A solution of the azepane-derived amino acid **3** (0.233 g, 0.65 mmol) in dry CH_2Cl_2 (10 mL) was treated with BOP (0.434 g, 0.98 mmol), H-Ala-Ala-NHR·HCl (0.98 mmol) and TEA (0.273 mL, 1.96 mmol). After being stirred at room temperature for 15 h, 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. Then, the residue was purified on a silica gel column, using the solvent system specified in each case.

Boc-Aze(Alloc)-Ala-Ala-NHMe (4). Solid: mp 92−95 °C (EtOAc/hexane); yield, 0.170 g, 51%; eluent, MeOH/CH_2Cl_2 (1:40); $[\alpha]_D^{20}$ = +18.2 (*c* 0.8, $CHCl_3$); HPLC t_R = 10.95 min (gradient A/B from 5:95 to 80:20 over 20 min); ^{1}H NMR (300 MHz, DMSO-d_6, two rotamers, Mr/mr = 1.2:1) δ 0.83 (d, 3H, *J* = 5.6, 3-CH_3, mr), 0.84 (d, 3H, *J* = 6.1, 3-CH_3, Mr), 1.21 (d, 3H, *J* = 6.6, α-CH_3, Ala), 1.23 (d, 3H, *J* = 6.6, α-CH_3, Ala), 1.41 (s, 9H, CH_3, Boc), 1.58 (m, 2H, 6-H), 1.72 (m, 1H, 5-H), 2.08 (m, 1H, 3-H), 2.30 (m, 1H, 5-H), 2.56 (d, 3H, *J* = 4.6, NCH_3), 3.03 (td, 1H, *J* = 13.0 and 6.0, 7-H, mr), 3.13 (td, 1H, *J* = 13.2 and 6.5, 7-H, Mr), 3.36 (m, 1H, 2-H), 3.44 (m, 1H, 2-H), 3.53 (m, 1H, 7-H), 4.15 (quint., 2H, *J* = 6.6, α-H, Ala), 4.53 (m, 2H, 1′-H, Alloc), 5.18 (ddt, 1H, *J* = 10.5, 3.0 and 1.7, 3′-H, Alloc), 5.26 (ddt, 1H, *J* = 17.2, 3.4 and 1.7, 3′-H, Alloc), 5.92 (ddt, 1H, *J* = 17.2, 10.5 and 5.0, 2′-H, Alloc), 6.95 (s, 1H, 4-NH, Mr), 7.02 (s, 1H, 4-NH, mr), 7.41 (m, 1H, N*H*CH_3, Mr), 7.48 (m, 1H, N*H*CH_3, mr), 7.85 (d, 1H, *J* = 6.6, α-NH, Ala^{i+3}), 8.00 (m, 1H, α-NH, Ala^{i+2}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.3 (3-CH_3), 17.3 (α-CH_3, Ala), 17.7 (α-CH_3, Ala), 20.1 (6-C, mr), 20.8 (6-C, Mr), 25.5 (NCH_3, Mr), 25.6 (NCH_3, mr), 28.1 (CH_3, Boc), 29.6 (5-C, mr), 29.9 (5-C, Mr), 40.6 (3-C), 45.3 (7-C, Mr), 45.4 (7-C, mr), 47.0 (2-C, mr), 47.6 (2-C, Mr), 48.2 (α-CH, Ala^{i+3}, Mr), 48.3 (α-CH, Ala^{i+3}, mr), 49.5 (α-CH, Ala^{i+2}), 63.3 (4-C, mr), 63.6 (4-C, Mr), 65.0 (1′-C, Alloc, Mr), 65.1 (1′-C, Alloc, mr), 78.8 (C, Boc, mr), 78.9 (C, Boc, Mr), 116.5 (3′-C, Alloc, Mr), 116.6 (3′-C, Alloc, mr), 133.6 (2′-C, Alloc), 155.1 and 155.6 (CO, Alloc and Boc, mr), 155.3 and 155.5 (CO, Alloc and Boc, Mr), 171.9 (CONH), 172.1 (CONH, Mr), 172.2 (CONH), 172.3 (CONH, mr); MS (ESI) *m*/*z* = 512.3 [M + H]$^+$, 1045.5 [2M + Na]$^+$. Elemental analysis calcd (%) for $C_{24}H_{41}N_5O_7$: C 56.34, H 8.08, N 13.69. Found (%): C 56.22, H 7.94, N 13.43.

Boc-Aze(Alloc)-Ala-Ala-NH^{i}Pr (5). Solid: mp 160−162 °C (EtOAc/hexane); yield, 0.312g, 88%; eluent, EtOAc:hexane (8:1); $[\alpha]_D^{20}$ = +27.3 (*c* 0.9, $CHCl_3$); HPLC t_R = 12.50 min (gradient A/B from 5:95 to 80:20 over 20 min); ^{1}H NMR (400 MHz, DMSO-d_6, two rotamers, Mr/mr = 1.3:1) δ 0.84 (d, 3H, *J* = 6.6, 3-CH_3, Mr), 0.86 (d, 3H, *J* = 6.6, 3-CH_3, mr), 1.02 (d, 6H, *J* = 7.3, CH_3, ^{i}Pr), 1.21 (d, 3H, *J* = 7.2, α-CH_3, Ala), 1.24 (d, 3H, *J* = 7.2, α-CH_3, Ala), 1.42 (s, 9H, CH_3, Boc), 1.48 (m, 1H, 6-H), 1.66 (m, 1H, 6-H), 1.73 (m, 1H, 5-H), 2.06 (m, 1H, 3-H), 2.34 (m, 1H, 5-H), 3.02 (td, 1H, *J* = 12.8 and 6.3, 7-H, mr), 3.12 (td, 1H, *J* = 13.8 and 6.7, 7-H, Mr), 3.29 (m, 1H, 2-H), 3.46 (d, 1H, *J* = 13.6, 2-H, Mr), 3.49 (d, 1H, *J* = 12.3, 2-H, mr), 3.59 (m, 1H, 7-H), 3.78 (oct, 1H, *J* = 7.3, CH, ^{i}Pr), 4.10 (quint., 2H, *J* = 7.2, α-H, Ala), 4.52 (m, 2H, 1′-H, Alloc), 5.17 (ddt, 1H, *J* = 10.4, 2.5 and 1.6, 3′-H, Alloc), 5.25 (ddt, 1H, *J* = 17.5, 4.8 and 1.6, 3′-H, Alloc), 5.91 (ddt, 1H, *J* = 17.5, 10.4 and 4.8, 2′-H, Alloc), 7.01 (s, 1H, 4-NH, Mr), 7.07 (s, 1H, 4-NH, mr), 7.15 (d, 1H, *J* = 7.3, N*H*^{i}Pr, Mr), 7.18 (d, 1H, *J* = 7.3, N*H*^{i}Pr, mr), 7.83 (d, 1H, *J* = 7.2, α-NH, Ala^{i+3}), 8.09 (m, 1H, α-NH, Ala^{i+2}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.3 (3-CH_3, mr), 13.4 (3-CH_3, Mr), 17.3 (α-CH_3, Ala), 17.7 (α-CH_3, Ala), 20.1 (6-C, mr), 20.8 (6-C, Mr), 22.1 (CH_3, ^{i}Pr), 22.2 (CH_3, ^{i}Pr), 28.1 (CH_3, Boc), 29.8 (5-C, mr), 30.1 (5-C, Mr), 40.0 (3-C), 40.6 (CH, ^{i}Pr), 45.2 (7-C, Mr), 45.4 (7-C, mr), 47.2 (2-C, mr), 47.8 (2-C, Mr), 48.4 (α-CH, Ala^{i+3}), 49.6 (α-CH, Ala^{i+2}), 63.6 (4-C), 64.9 (1′-C, Alloc, Mr), 65.0 (1′-C, Alloc, mr), 78.9 (C, Boc, mr), 80.0 (C, Boc, Mr), 116.5 (3′-C, Alloc), 133.6 (2′-C, Alloc), 155.1 and 155.7 (CO, Alloc and Boc, Mr), 155.3 and 155.7 (CO, Alloc and Boc, mr), 170.8, 172.1, and 174.4 (CONH); MS (ESI) *m*/*z* = 540.3 [M + H]$^+$, 1101.7 [2M + Na]$^+$. Elemental analysis calcd (%) for $C_{26}H_{45}N_5O_7$: C 57.87, H 8.40, N 12.98. Found (%): C 57.85, H 8.76, N 12.85.

Boc-Aze-Ala-Ala-NH^{i}Pr (6). A solution of tripeptide **5** (0.048 g, 0.09 mmol) in dry CH_2Cl_2 (3 mL) under Ar atmosphere was treated with $Pd(PPh_3)_4$ (0.010 g, 0.009 mmol) and $PhSiH_3$ (0.110 mL, 0.89 mmol). After being stirred at room temperature for 1 h, the solvent was evaporated to dryness, and the residue was dissolved in H_2O and purified by reverse-phase flash chromatography using a gradient CH_3CN/H_2O (0.05% TFA) from 0:100 to 100:0. Solid: mp 82−85 °C (MeOH); yield, 0.035 g, 69%; $[\alpha]_D^{20}$ = −1.2 (*c* 1.0, MeOH); HPLC t_R = 1.33 min (gradient A/B from 2:98 to 5:95 over 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92 (d, 3H, *J* = 7.1, 3-CH_3), 1.03 (d, 6H, *J* = 7.0, CH_3, ^{i}Pr), 1.20 (d, 3H, *J* = 6.9, α-CH_3, Ala^{i+2}), 1.24 (d, 3H, *J* = 7.3, α-CH_3, Ala^{i+3}), 1.41 (s, 9H, CH_3, Boc), 1.68 (m, 2H, 6-H), 2.14 (m, 2H, 5-H), 2.54 (m, 1H, 3-H), 2.91 (m, 1H, 7-H), 3.12 (m, 2H, 2-H), 3.16 (m, 1H, 7-H), 3.78 (oct, 1H, *J* = 7.0, CH, ^{i}Pr), 4.12 (quint., 1H, *J* = 7.3, α-H, Ala^{i+3}), 4.15 (quint., 1H, *J* = 6.9, α-H, Ala^{i+2}), 7.30 (s, 1H, 4-NH), 7.34 (bs, 1H, N*H*^{i}Pr), 7.84 (d, 1H, *J* = 6.9, α-NH, Ala^{i+3}), 8.23 (bs, 1H, α-NH, Ala^{i+2}), 8.40 (bs, 1H, 1-H), 8.84 (bs, 1H, 1-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.4 (3-CH_3), 17.4 (α-CH_3, Ala^{i+3}), 18.0 (α-CH_3, Ala^{i+2}), 19.4 (6-C), 22.2 (CH_3, ^{i}Pr), 22.3 (CH_3, ^{i}Pr), 28.1 (CH_3, Boc), 31.4 (5-C), 36.5 (3-C), 40.4 (CH, ^{i}Pr), 46.0 (7-C), 47.5 (2-C), 48.4 (α-CH, Ala^{i+3}), 49.5 (α-CH, Ala^{i+2}), 63.4 (4-C), 79.2 (C, Boc), 155.5 (CO, Boc), 170.9, 171.8, and 174.3 (CONH); MS (ESI) *m*/*z* = 456.3 [M − TFA + H]$^+$, 911.7 [2M − TFA + Na]$^+$. Elemental analysis calcd (%) for $C_{24}H_{42}N_5O_7F_3$: C 50.61, H 7.43, N 12.29. Found (%): C 50.72, H 7.14, N 12.17.

Ac-Aze(Alloc)-Ala-Ala-NH^{i}Pr (7). Tripeptide derivative **5** (0.045 g, 0.08 mmol) was dissolved in a solution of HCl/EtOAc (4 mL, 3.1 M), and the solution was stirred at room temperature for 4 h. After evaporation of the solvent, the crude mixture was dissolved in dry CH_2Cl_2 (4 mL) and TEA (0.012 mL, 0.08 mmol), and propylene oxide (0.088 mL, 1.25 mmol) and Ac−Cl (0.007 mL, 0.10 mmol) were added. The solution was stirred at room temperature for 18 h, the solvent was evaporated to dryness, and the residue was purified by chromatography on silica gel using MeOH:CH_2Cl_2 (1:10). Solid: mp 118−121 °C, EtOAc:hexane); yield, 0.031 g, 78%; $[\alpha]_D^{20}$ = +25.6 (*c* 0.8, MeOH); HPLC t_R = 8.56 min (gradient A/B from 5:95 to 80:20 over 20 min); ^{1}H NMR (400 MHz, DMSO-d_6, two rotamers, Mr/mr = 1.1:1) δ 0.88 (d, 3H, *J* = 6.9, 3-CH_3, Mr), 0.90 (d, 3H, *J* = 6.8, 3-CH_3, mr), 1.00 (d, 6H, *J* = 6.8, CH_3, ^{i}Pr, Mr), 1.02 (d, 6H, *J* = 6.8, CH_3, ^{i}Pr, mr), 1.22 (d, 3H, *J* = 7.3, α-CH_3, Ala^{i+3}), 1.24 (d, 3H, *J* = 7.3, α-CH_3, Ala^{i+2}), 1.39 (m, 1H, 6-H), 1.67 (m, 2H, 5-H, 6-H), 1.96 (m, 1H, 3-H), 2.01 (s, 3H, CH_3, Ac), 2.46 (m, 1H, 5-H), 2.97 (td, 1H, *J* = 12.8 and 6.0, 7-H, mr), 3.06 (td, 1H, *J* = 13.0 and 6.6, 7-H, Mr), 3.23 (m, 1H, 2-H), 3.52 (m, 1H, 2-H), 3.64 (m, 1H, 7-H), 3.77 (oct, 1H, *J* = 6.8, CH, ^{i}Pr), 4.04 (quint., 1H, *J* = 7.3, α-H, Ala^{i+3}), 4.11 (quint., 1H, *J* = 7.3, α-H, Ala^{i+2}, Mr), 4.12 (quint., 1H, *J* = 7.3, α-H, Ala^{i+2}, mr), 4.53 (m, 2H, 1′-H, Alloc), 5.16 (dq, 1H, *J* = 10.3 and 1.7, 3′-H, Alloc, Mr), 5.17 (dq, 1H, *J* = 10.3 and 1.7, 3′-H, Alloc, mr), 5.24 (dq, 1H, *J* = 17.3 and 1.7, 3′-H, Alloc, Mr), 5.26 (dq, 1H, *J* = 17.3 and 1.7, 3′-H, Alloc, mr),5.93 (ddt, 1H, *J* = 17.3, 10.3 and 5.0, 2′-H, Alloc), 7.07 (d, 1H, *J* = 6.8, N*H*^{i}Pr, mr), 7.10 (d, 1H, *J* = 6.8, N*H*^{i}Pr, Mr), 7.73 (d, 1H, *J* = 7.3, α-NH, Ala^{i+3}), 7.80 (s, 1H, 4-NH, Mr), 7.82 (s, 1H, 4-NH, mr), 8.13 (d, 1H, *J* = 7.1, α-NH, Ala^{i+2}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.4 (3-CH_3, Mr), 13.5 (3-CH_3, mr), 16.8 (α-CH_3, Ala^{i+2}), 17.4 (α-CH_3, Ala^{i+3}), 20.3 (6-C, mr), 20.9 (6-C, Mr), 22.1 (CH_3, ^{i}Pr), 22.2 (CH_3, ^{i}Pr), 23.4 (CH_3, Ac, Mr), 23.5 (CH_3, Ac, mr), 29.4 (5-C, mr), 29.7 (5-C, Mr), 40.4 (CH, ^{i}Pr), 41.1 (3-C), 45.2 (7-C, Mr), 45.4 (7-C, mr), 47.4 (2-C, mr), 48.1 (2-C, Mr), 48.6 (α-CH, Ala^{i+3}), 49.6 (α-CH, Ala^{i+2}), 64.0 (4-C), 65.0 (1′-C, Alloc), 116.5 (3′-C, Alloc), 133.6 (2′-C, Alloc), 155.1 (CO, Alloc, mr), 155.3 (CO, Alloc, Mr), 171.0, 171.8 (mr), 171.9 (Mr), 172.1 and 173.9 (CONH); MS (ESI) *m*/*z* = 482.3 [M + H]$^+$, 985.7 [2M + Na]$^+$. Elemental analysis calcd (%) for $C_{23}H_{39}N_5O_6$: C 57.36, H 8.16, N 14.54. Found (%): C 57.45, H 8.01, N 14.64.

Boc-Aze(Alloc)-Ala-NHMe (8). A solution of the azepane-derived amino acid **3** (0.070 g, 0.20 mmol) in dry CH_2Cl_2 (7 mL) was treated with BOP (0.133 g, 0.30 mmol), H-Ala-NHMe·HCl (0.041 g, 0.30 mmol) and TEA (0.083 mL, 0.60 mmol). After being stirred at room temperature for 15 h, the solution was washed successively with citric acid (10%), $NaHCO_3$ (10%), H_2O and brine. The organic phase was

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dried over Na_2SO_4 and evaporated to dryness. Then, the residue was purified on a silica gel column, using EtOAc:hexane (8:1). Solid: mp 141−144 °C (MeOH); yield, 0.067 g, 78%. $[\alpha]_D^{20}$ = +11.0 (*c* 1.3, $CHCl_3$); HPLC t_R = 10.99 min (gradient A/B from 5:95 to 80:20 over 20 min); ^{1}H NMR (400 MHz, DMSO-d_6, two rotamers, Mr/mr = 1.1:1) δ 0.80 (d, 3H, *J* = 6.8, 3-CH_3, Mr), 0.82 (d, 3H, *J* = 6.8, 3-CH_3, mr), 1.21 (d, 3H, *J* = 7.0, α-CH_3, Ala), 1.42 (s, 9H, CH_3, Boc), 1.46 (m, 1H, 6-H), 1.63 (m, 1H, 6-H), 1.68 (m, 1H, 5-H), 2.07 (m, 1H, 3-H), 2.30 (m, 1H, 5-H), 2.56 (d, 3H, *J* = 4.5, NCH_3), 3.01 (td, 1H, *J* = 13.2 and 6.8, 7-H, mr), 3.11 (td, 1H, *J* = 13.5 and 6.5, 7-H, Mr), 3.29 (m, 1H, 2-H), 3.45 (m, 1H, 2-H), 3.58 (m, 1H, 7-H), 4.23 (quint., 1H, *J* = 7.0, α-H, Ala, Mr), 4.24 (quint., 1H, *J* = 7.0, α-H, Ala, mr), 4.52 (m, 2H, 1′-H, Alloc), 5.18 (dq, 1H, *J* = 10.5 and 1.5, 3′-H, Alloc), 5.26 (ddt, 1H, *J* = 17.2, 3.5 and 1.5, 3′-H, Alloc), 5.92 (ddt, 1H, *J* = 17.2, 10.5 and 5.1, 2′-H, Alloc), 6.94 (s, 1H, 4-NH, Mr), 7.00 (s, 1H, 4-NH, mr), 7.62 (bs, 1H, N*H*CH_3), 7.80 (d, 1H, *J* = 7.0, α-NH, Ala, Mr), 7.83 (d, 1H, *J* = 7.0, α-NH, Ala, mr); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.2 (3-CH_3, mr), 13.3 (3-CH_3, Mr), 17.6 (α-CH_3, Ala), 20.1 (6-C, Mr), 20.8 (6-C, mr), 25.5 (NCH_3), 28.2 (CH_3, Boc), 30.0 (5-C), 40.8 (3-C), 45.3 (7-C, Mr), 45.5 (7-C, mr), 47.1 (2-C, Mr), 47.7 (2-C, mr), 48.2 (α-CH, Ala), 63.7 (4-C), 65.0 (1′-C, Alloc), 78.8 (C, Boc, Mr), 78.9 (C, Boc, mr), 116.5 (3′-C, Alloc, mr), 116.7 (3′-C, Alloc, Mr), 133.6 (2′-C, Alloc), 155.4 and 155.8 (CO, Alloc and Boc, Mr), 155.2 and 155.7 (CO, Alloc and Boc, mr), 172.5 and 173.1 (CONH); MS (ESI) *m*/*z* = 441.2 $[M + H]^+$, 463.3 $[M + Na]^+$, 881.7 $[2M + H]^+$, 903.5 $[2M + Na]^+$. Elemental analysis calcd (%) for $C_{21}H_{36}N_4O_6$: C 57.25, H 8.24, N 12.72. Found (%): C 57.08, H 8.07, N 12.80.

Boc-Ala-Aze(Alloc)-Ala-NHMe (9). Dipeptide derivative **5** (0.035 g, 0.08 mmol) was dissolved in a solution of HCl/EtOAc (4 mL, 3.1 M), and the solution was stirred at room temperature for 4 h. After evaporation of the solvent, the crude mixture was dissolved in dry THF (6 mL), and TEA (0.030 mL, 0.22 mmol), Boc-Ala-OH (0.025 g, 0.13 mmol) and BOP (0.059 g, 0.13 mmol) were added. After being stirred at 65 °C for 48 h, the solvent was evaporated to dryness, and the residue was dissolved in EtOAc. Then, 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 on a silica gel column, using EtOAc:hexane (10:1). Solid: mp 73−76 °C (MeOH); yield, 0.019 g, 47%; $[\alpha]_D^{20}$ = +27.6 (*c* 0.9, $CHCl_3$); HPLC t_R = 11.28 min (gradient A/B from 5:95 to 80:20 over 20 min); ^{1}H NMR (400 MHz, DMSO-d_6, two rotamers, A/B = 1:1) δ 0.82 (d, 3H, *J* = 6.3, 3-CH_3, A), 0.83 (d, 3H, *J* = 6.6, 3-CH_3, B), 1.25 (d, 3H, *J* = 7.6, α-CH_3, Ala^{i+1}), 1.26 (d, 3H, *J* = 6.9, α-CH_3, Ala^{i+3}. A), 1.27 (d, 3H, *J* = 6.9, α-CH_3, Ala^{i+3}, B), 1.39 (s, 9H, CH_3, Boc), 1.43 (m, 1H, 6-H), 1.64 (m, 1H, 6-H), 1.71 (m, 1H, 5-H), 2.03 (m, 1H, 3-H), 2.48 (m, 1H, 5-H), 2.57 (d, 3H, *J* = 4.7, NCH_3), 2.97 (m, 1H, 7-H, A), 3.08 (td, 1H, *J* = 12.9 and 6.1, 7-H, B), 3.23 (m, 1H, 2-H), 3.52 (m, 1H, 2-H), 3.60 (m, 1H, 7-H), 3.97 (m, 1H, α-H, Ala^{i+1}), 4.17 (quint., 1H, *J* = 6.9, α-H, Ala^{i+3}), 4.53 (m, 2H, 1′-H, Alloc), 5.18 (ddt, 1H, *J* = 10.5, 3.2 and 1.7, 3′-H, Alloc), 5.26 (ddt, 1H, *J* = 17.3, 3.2 and 1.7, 3′-H, Alloc), 5.92 (ddt, 1H, *J* = 17.3, 10.5 and 5.1, 2′-H, Alloc), 7.31 (d, 1H, *J* = 5.2, α-NH, Ala^{i+1}, A), 7.32 (d, 1H, *J* = 5.0, α-NH, Ala^{i+1}, B), 7.46 (q, 1H, *J* = 4.7, N*H*CH_3), 7.48 (s, 1H, 4-NH, A), 7.49 (s, 1H, 4-NH, B), 7.58 (d, 1H, *J* = 6.9, α-NH, Ala^{i+3}, A), 7.59 (d, 1H, *J* = 6.9, α-NH, Ala^{i+3}, B); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.3 (3-CH_3, A), 13.4 (3-CH_3, B), 16.4 (α-CH_3, Ala), 16.9 (α-CH_3, Ala, A), 17.5 (α-CH_3, Ala, B), 20.0 (6-C, A), 20.7 (6-C, B), 25.6 (NCH_3), 28.1 (CH_3, Boc), 29.5 (5-C, A), 29.9 (5-C, B), 41.2 (3-C), 45.2 (7-C, A), 45.4 (7-C, B), 47.4 (2-C, A), 48.0 (2-C, B), 48.7 (α-CH, Ala^{i+3}), 51.6 (α-CH, Ala^{i+1}, A), 51.7 (α-CH, Ala^{i+1}, B), 64.1 (4-C), 65.1 (1′-C, Alloc), 79.0 (C, Boc), 116.6 (3′-C, Alloc, A), 116.8 (3′-C, Alloc, B), 133.5 (2′-C, Alloc, A), 133.6 (2′-C, Alloc, B), 155.0 and 156.3 (CO, Alloc and Boc, A), 155.2 and 156.3 (CO, Alloc and Boc, B), 172.3, 172.4, and 174.9 (CONH); MS (ESI) *m*/*z* = 512.3 $[M + H]^+$, 1045.7 $[2M + Na]^+$. Elemental analysis calcd (%) for $C_{24}H_{41}N_5O_7$: C 56.34, H 8.08, N 13.69. Found (%): C 56.26, H 8.21, N 13.54.

Preparation of Single Crystals for X-ray Diffraction Analysis. Pure compound 7 (8 mg) was dissolved in EtOAc (4 mL), and the mixture was put in a crystallizing dish, resulting in spontaneous crystallization after 7 days at room temperature in a closed jar, saturated with hexane.

■ ASSOCIATED CONTENT

Supporting Information

Crystallographic data (CIF) of derivative **7**, computational results and copies of the ^{1}H and ^{13}C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Stites, W. E. *Chem. Rev.* **1997**, *97*, 1233.
(2) Pérez de Vega, M. J.; Martín-Martínez, M.; González-Muñiz, R. *Curr. Top. Med. Chem.* **2007**, *7*, 33.
(3) Meireles, L. M. C.; Mustata, G. *Curr. Top. Med. Chem.* **2011**, *11*, 248.
(4) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers* **2001**, *60*, 396.
(5) Maity, P.; Koenig, B. *Biopolymers* **2008**, *90*, 8.
(6) Yokum, T. S.; Gauthier, T. J.; Hammer, R. P.; McLaughlin, M. L. *J. Am. Chem. Soc.* **1997**, *119*, 1167.
(7) Jiménez, A. I.; Cativiela, C.; Gómez-Catalán, J.; Pérez, J. J.; Aubry, A.; París, M.; Marraud, M. *J. Am. Chem. Soc.* **2000**, *122*, 5811.
(8) Benedetti, E.; DiBlasio, B.; Iacovino, R.; Menchise, V.; Saviano, M.; Pedone, C.; Bonora, G. M.; Ettorre, A.; Graci, L.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2023.
(9) Morera, E.; Lucente, G.; Ortar, G.; Nalli, M.; Mazza, F.; Gavuzzo, E.; Spisani, S. *Bioorg. Med. Chem.* **2002**, *10*, 147.
(10) (a) Maity, P.; Zabel, M.; König, B. *J. Org. Chem.* **2007**, *72*, 8046. (b) Grauer, A. A.; Cabrele, C.; Zabel, M.; König, B. *J. Org. Chem.* **2009**, *74*, 3718.
(11) Ousaka, N.; Tani, N.; Sekiya, R.; Kuroda, R. *Chem. Commun.* **2008**, 2894.
(12) (a) Toniolo, C.; Crisma, M.; Formaggio, F. *Biopolymers* **1998**, *47*, 153. (b) Peggion, C.; Jost, M.; De Borggraeve, W. M.; Crisma, M.; Formaggio, F.; Toniolo, C. *Chem. Biodiversity* **2007**, *4*, 1256. (c) Schreier, S.; Bozelli, J.; Marín, N.; Vieira, R.; Nakaie, C. *Biophys. Rev* **2012**, *4*, 45.
(13) (a) Toniolo, C.; Valente, E.; Formaggio, F.; Crisma, M.; Pilloni, G.; Corvaja, C.; Toffoletti, A.; Martinez, G. V.; Hanson, M. P.; Millhauser, G. L.; George, C.; Flippen-Anderson, J. L. *J. Pept. Sci.* **1995**, *1*, 45. (b) Smythe, M. L.; Nakaie, C. R.; Marshall, G. R. *J. Am. Chem. Soc.* **1995**, *117*, 10555. (c) Flippen-Anderson, J. L.; George, C.; Valle, G.; Valente, E.; Bianco, A.; Formaggio, F.; Crisma, M.; Toniolo, C. *Int. J. Pept. Protein Res.* **1996**, *47*, 231. (d) Hanson, P.; Martinez, G.; Millhauser, G.; Formaggio, F.; Crisma, M.; Toniolo, C.; Vita, C. *J. Am. Chem. Soc.* **1996**, *118*, 271. (e) Hanson, P.; Anderson, D. J.; Martinez, G.; Millhauser, G.; Formaggio, F.; Crisma, M.; Toniolo, C.; Vita, C. *Mol. Phys.* **1998**, *95*, 957.

(14) Crisma, M.; Deschamps, J. R.; George, C.; Flippen-Anderson, J. L.; Kaptein, B.; Broxterman, Q. B.; Moretto, A.; Oancea, S.; Jost, M.; Formaggio, F.; Toniolo, C. *J. Pept. Res.* **2005**, *65*, 564.
(15) Núñez-Villanueva, D.; Bonache, M. A.; Infantes, L.; García-López, M. T.; Martín-Martínez, M.; González-Muñiz, R. *J. Org. Chem.* **2011**, *76*, 6592.
(16) Ousaka, N.; Sato, T.; Kuroda, R. *J. Am. Chem. Soc.* **2009**, *131*, 3820.
(17) (a) Kuwano, R.; Takahashi, M.; Ito, Y. *Tetrahedron Lett.* **1998**, *39*, 1017. (b) Gerona-Navarro, G.; Bonache, M. A.; Alías, M.; Pérez de Vega, M. J.; García-López, M. T.; López, P.; Cativiela, C.; González-Muñiz, R. *Tetrahedron Lett.* **2004**, *45*, 2193.
(18) Ac indicates acetyl group; Xaa, Yaa and Zaa indicates an amino acid. Aze indicates (3*R*,4*S*)-4-amino-4-carbonyl-3-methylazepane.
(19) For clarity, type III β-turn is indicated: Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425. This type of turn is now considered to correspond to a turn of a 3_{10} helix: Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167.
(20) (a) Kessler, H. *Angew. Chem., Int. Ed.* **1982**, *21*, 512. (b) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, *1999*, 389. (c) Baeza, J. L.; Gerona-Navarro, G.; Thompson, K.; Pérez de Vega, M. J.; aInfantes, L.; García-López, M. T.; González-Muñiz, R.; Martín-Martínez, M. *J. Org. Chem.* **2009**, *74*, 8203.
(21) CCDC 887956 contains the supplementary crystallographic data for compound **7**. This data can be obtained free of charge from The Cambridge Crystallographic Data Center via http://www.ccdc.cam.ac.uk/data_request/cif.
(22) A marked helicogenic effect in short Ala-Aib pentapeptides was recently described for a related 1H-Azepine-4-amino-4-carboxylic residue: Pellegrino, S.; Contini, A.; Clerici, F.; Gori, A.; Nava, D.; Gelmi, M. L. *Chem.—Eur. J.* **2012**, *18*, 8705.