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RESEARCH ARTICLE

Heterochiral Ala / (α Me)Aze sequential oligopeptides: synthesis and conformational study

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(Abstract)

α -Amino acid residues with a ϕ, ψ constrained conformation are known to significantly bias the peptide backbone 3D-structure. An intriguing member of this class of compounds is (α Me)Aze, characterized by an N $^{\alpha}$ -alkylated four-membered ring and C $^{\alpha}$ -methylation. We have already reported that (*S*)-(α Me)Aze, when combined with (*S*)-Ala in the *homo-chiral* dipeptide sequential motif -(*S*)-(α Me)Aze-(*S*)-Ala-, tends to generate the unprecedented γ -bend ribbon conformation, as formation of a regular, fully intramolecularly H-bonded γ -helix is precluded, due to the occurrence of a tertiary amide bond every two residues. In this work, we have expanded this study to the preparation and 3D-structural analysis of the *hetero-chiral* -(*S*)-Ala-(*R*)-(α Me)Aze- sequential oligopeptides from the dimer to hexamer. Our conformational results show that members of this series may fold easily either in type-II β -turns or in γ -turns depending on the experimental conditions.

KEYWORDS

azetidines, hetero-chiral sequences, peptide conformation, peptide synthesis, X-ray diffraction, β/γ -turns

1 | INTRODUCTION

Main-chain reversals (turns) in naturally-occurring and synthetic polypeptide molecules are ubiquitous.^{1,2} Among them (α -³, β -⁴⁻⁷, γ -⁸⁻¹², δ -¹³, ε -¹⁴, and π -¹⁵ turns), β -turns are by far the most extensively investigated both experimentally and computationally. All other types of turns, although drastically less frequently reported, are currently the focus of an increasing number of publications, in particular γ -turns.¹² Except for the case of α - and β -turns (a repetition of one type of α -turn generates the classical α -helix¹⁶ while that of some β -turns produces either the 3_{10} -helix,¹⁷⁻²⁰ also well known, particularly in the peptide structural biochemistry literature, or β -turn ribbons²¹⁻²³), incipient helices or ribbon-like stretches^{23,24} arising from all other types of turns have attracted much less interest from chemists.

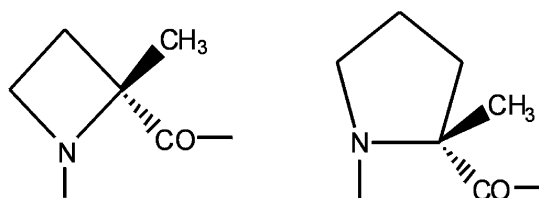


Figure 1. Chemical structures of (*R*)-(α Me)Aze (left) and (*R*)-(α Me)Pro (right).

Recently, with the aim of reducing this gap, we focused our attention on γ -turns.^{12,24} We wished in particular to discover and investigate in detail non-coded α -amino acids with a high propensity to fold into single turns, and possibly offering a good chance to create new, longer ordered secondary structures. To this end, we especially considered two C^α -tetrasubstituted α -amino acids as potentially very promising building blocks, the chiral (α Me)Aze²⁴⁻³¹ (Figure 1) and the achiral 2-aminoadamantane-2-carboxylic acid.³² In particular, by examining a series of repeating, homo-chiral, sequential $-(S)-(\alpha$ Me)Aze- (S) -Ala- dipeptides, we found a novel stable peptide conformation, the γ -bend ribbon structure,²⁴ characterized by only half of the $C=O\dots H-N$ intramolecular H-bonds typical of the γ -helix¹² because the strong γ -bend inducer (α Me)Aze, which forms Xxx- $(\alpha$ Me)Aze tertiary amides, lacks the N-H donor function.

Here, we extended the investigation on this repeating, sequential dipeptide series by describing our results on the solution synthesis and in-depth 3D-structural characterization of the - (S)-Ala-(R)-(α Me)Aze- *hetero*-chiral oligomers to the hexapeptide level. Some results reported in this article have been presented in the Proceedings of a recent peptide symposium.³³

Abbreviations: Adm, 2-aminoadamantane-2-carboxylic acid; Aib, α -aminoisobutyric acid; (α Me)Aze, C ^{α} -methyl azetidine carboxylic acid; (α Me)Pro, C ^{α} -methyl proline; Boc, *tert*-butyloxycarbonyl; CD, circular dichroism; c₃Dip, 2,2-diphenyl-1-aminocyclopropane-1-carboxylic acid; DIPEA, diisopropylethylamine; DMF, N,N-dimethylformamide; Et₂O, diethyl ether; EtOAc, ethyl acetate; FT-IR, Fourier-transform infrared absorption; HATU, 1-[*bis*(dimethylamino)methylene]-1-*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate; *i*BuCO, *isobutanoyl*; NHiPr, *isopropylamino*; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; OMe, methoxy; TFE, 2,2,2-trifluoroethanol; UV, ultraviolet.

2 | EXPERIMENTAL

2.1 | Peptide synthesis and characterization

2.1.1 | General methods. ^1H and ^{13}C spectra were recorded on a Bruker Avance spectrometer at 200 or 300 and 50 or 75 MHz, respectively. Chemical shifts are reported in ppm from tetramethylsilane (TMS). Optical rotations were determined with a Perkin Elmer 341 instrument. All reactions were carried out under argon. Column chromatography was performed on a silica gel 230-400 mesh by using various mixtures of solvents. TLCs were run on Merck Kieselgel 60F₂₅₄ plates. Melting points are uncorrected. Infrared absorption spectra were recorded on a Nicolet iS 10 (SMART iTR diamond ATR) spectrophotometer. High-resolution mass spectra (HR-MS) were obtained on a Water Micromass Q-ToF Micro instrument.

2.1.2 | Synthesis

A practical synthesis of the enantiopure Boc-(*R*)-(αMe)Aze-OMe (**1**) was published by the Versailles group.³⁰

Boc-(*S*)-Ala-(*R*)-(αMe)Aze-OMe (2**)**

Boc-(*R*)-(αMe)Aze-OMe (**1**) (1 g, 4.37 mmol) was dissolved in 1.7 M HCl solution in Et₂O (25 mL). After overnight stirring, a precipitate formed. Solvent was removed under vacuum. The HCl · H-(*R*)-(αMe)Aze-OMe salt was washed with Et₂O at 0°C (1.029 g, 4.10 mmol, 94%). To this residue THF (20 mL) was added and the resulting suspension was cooled to 0°C. Boc-(*S*)-Ala-OH (0.935 g, 4.92 mmol), HATU (1.87 g, 4.92 mmol), and DIPEA (2.65 mL, 15.17 mmol) were added. After stirring for 72 h, THF was removed by evaporation and the residue was taken up in CH₂Cl₂ (80 mL). The organic layer was successively washed with a saturated aqueous solution of sodium hydrogen carbonate (30 mL) and brine (30 mL). The solvent was dried over magnesium sulfate and evaporated to dryness. The residue was purified by chromatography (CH₂Cl₂/MeOH 98:2). The title compound **2** was obtained as an oil (1.101 g, 84% from **1**). R_f : 0.30 (CH₂Cl₂/MeOH 95:5). $[\alpha]_D^{20}$: +28.4 (c 1.03, CH₂Cl₂), ^1H NMR (300 MHz, CDCl₃): two rotamers (8:2 ratio), δ = 1.28 (d, J = 6.9 Hz, 3H, CH₃), 1.40 (minor rotamer) and 1.43 (major rotamer) (s, 9H, *t*Bu), 1.70 (major rotamer) and 1.85 (minor rotamer) (s, 3H, CH₃), 2.13-2.22 (m, 1H, H-3), 2.39-2.47 (m, 1H, H-3'), 2.50-2.59 (m, 1H, H-3' minor rotamer), 3.75 (major rotamer), 3.78 (minor rotamer) (s, 3H, OMe), 3.83-3.99 (m, 2H, H-4, H-4' minor rotamer), 4.03-4.10 (m, 1H, H-4 major rotamer), 4.15-4.24 (m, 1H, H α),

4.28-4.36 (m, 1H, H-4' major rotamer), 5.07 (d, $J = 9.1$ Hz, 1H, NH Boc minor rotamer), 5.31 (d, $J = 8.2$ Hz, 1H, NH Boc major rotamer) ppm. ^{13}C NMR (75 MHz, CDCl_3): major rotamer $\delta = 18.6$, 21.8, (CH_3), 28.3 (*t*Bu), 29.0 (CH_2), 45.9 (C^α), 46.8 (CH_2), 52.6 (CH_3), 67.4, 79.5 (Cq), 155.1, 172.1, 172.3 (CO) ppm. IR (ATR): ν_{max} 3305, 2977, 2934, 2890, 1740, 1708, 1648, 1501, 1448, 1427, 1365, 1289, 1247, 1160, 1131, 1061, 861, 778 cm^{-1} ; HRMS (ESI, +ve): calculated for $\text{C}_{14}\text{H}_{25}\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+$: 301.1763; found 301.1753.

Boc-(S)-Ala-(R)-(α Me)Aze-OH (3)

To a solution of dipeptide **2** (0.165 g, 0.55 mmol) in THF (4 mL) and MeOH (2 mL) was added a solution of sodium hydroxide (0.044 g, 1.10 mmol) in water (2 mL). After stirring at 60°C for 4 hours, water (50 mL) was added to the reaction mixture and the organic solvents were evaporated. The remaining aqueous solution was washed with Et_2O (4×10 mL) and then acidified to pH = 1 with a 0.5 M HCl aqueous solution (5 mL). The aqueous layer was extracted with EtOAc (4×15 mL). The organic layer was dried over magnesium sulfate and evaporated to dryness. The residue was purified by chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 90:10). The title compound **3** was obtained as a solid (0.127 g, 81% from **2**). R_f : 0.60 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 80:20). Mp: 175°C. $[\alpha]_{\text{D}}^{20}$: + 37 (c 1.0, CH_2Cl_2), ^1H NMR (300 MHz, CD_3OD): two rotamers (80:20 ratio), $\delta = 1.33$ (d, $J = 6.9$ Hz, 3H, CH_3), 1.44 (minor rotamer) and 1.48 (major rotamer) (s, 9H, *t*Bu), 1.72 (major rotamer) and 1.82 (minor rotamer) (s, 3H, CH_3), 2.16-2.24 (m, 1H, H-3), 2.54-2.63 (m, 1H, H-3'), 3.81-3.93 (m, 0.4H, H-4 minor rotamer) and 4.16-4.30 (m, 2.7H, H-4 major rotamer, H-4', H α) ppm. ^{13}C NMR (75 MHz, CDCl_3): major rotamer $\delta = 18.5$, 23.9 (CH_3), 29.6 (*t*Bu), 30.2, 48.2 (CH_2), 49.1 (C^α), 73.3, 81.7 (Cq), 158.0, 174.8 (CO) ppm. IR (ATR): ν_{max} 3317, 2974, 2931, 2891, 1706, 1612, 1455, 1404, 1388, 1365, 1426, 1051 cm^{-1} ; HRMS (ESI, +ve): calculated for $\text{C}_{13}\text{H}_{22}\text{N}_2\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$: 309.1426; found 309.1424.

Boc-[(S)-Ala-(R)-(α Me)Aze]₂-OMe (4)

Dipeptide **2** (0.150 g, 0.50 mmol) was dissolved in 1.7 M HCl solution in Et_2O (5 mL). After overnight stirring, a precipitate formed. The solvent was removed under vacuum. To the dipeptide hydrochloride salt THF (5 mL) was added and the resulting suspension was cooled to 0°C. The dipeptide acid **3** (0.148 g, 0.52 mmol), HATU (0.228 g, 0.60 mmol), and DIPEA (0.320 mL, 1.85 mmol) were added. After overnight stirring, THF was removed by evaporation and the residue was taken up in EtOAc (100 mL). The organic layer was successively washed with a saturated aqueous solution of sodium hydrogen carbonate (30 mL), and brine (30 mL). The solvent was dried over magnesium sulfate and evaporated to dryness. The residue was purified by chromatography

(CH₂Cl₂/MeOH 92.5:2.5). The title compound **4** was obtained as white crystals (0.107 g, 41% from **2**). R_f : 0.30 (CH₂Cl₂/MeOH 95:5). Mp : 130°C. $[\alpha]_D^{20}$: + 97 (*c* 1.0, CH₂Cl₂), ¹H NMR (300 MHz, CDCl₃) : two rotamers (80:20 ratio), δ = 1.29-1.36 (m, 6H, CH₃), 1.42 (major rotamer) and 1.44 (minor rotamer) (s, 9H, *t*Bu), 1.71 (s, 3H, CH₃), 1.72 (s, 3H, CH₃), 2.07-2.22 (m, 1.8 H, 2 × H-3 major rotamer, H-3 minor rotamer), 2.37-2.45 (m, 1H, H-3 minor rotamer, H-3' major rotamer), 2.51-2.60 (m, 0.2 H, H-3' minor rotamer), 2.72-2.81 (m, 1H, H-3' major rotamer, H-3' minor rotamer), 3.75 (major rotamer) and 3.80 (minor rotamer) (s, 3H, OMe), 3.97-4.17 (m, 4H, 2 X H-4, H-4', Hα), 4.29-4.37 (m, 1H, H-4'), 4.40-4.50 (m, 1H, Hα), 5.47 (bs, 1H, NH Boc), 8.25-8.38 (m, 1H, NH amide) ppm. ¹³C NMR (75 MHz, CDCl₃): major rotamer δ = 17.5, 17.9, 21.8, 22.8 (CH₃), 27.5 (CH₂), 28.3 (*t*Bu), 45.3 (C^α), 45.7 (CH₂), 46.4 (C^α), 47.0 (CH₂), 52.5 (CH₃), 67.5, 71.1, 79.7 (Cq), 155.0, 171.3, 172.4, 172.8, 173.0 (CO) ppm. IR (ATR): ν_{max} 3297, 2978, 2933, 1740, 1691, 1646, 1531, 1458, 1433, 1360, 1290, 1245, 1160, 1131, 1004 cm⁻¹; HRMS (ESI, +ve): calculated for C₂₂H₃₇N₄O₇ [M+H]⁺: 469.2662; found 469.2665.

Boc-[(S)-Ala-(R)-(αMe)Aze]₃-OMe (5)

Tetrapeptide **4** (0.060 g, 0.128 mmol) was dissolved in 1.7 M HCl solution in Et₂O (5 mL). After overnight stirring, a precipitate formed. The solvent was removed under vacuum. To the tetrapeptide hydrochloride salt THF (5 mL) was added and the resulting suspension was cooled to 0°C. The dipeptide acid **3** (0.040 g, 0.139 mmol), HATU (0.054 g, 0.142 mmol), and DIPEA (0.083 mL, 0.473 mmol) were added. After overnight stirring, THF was removed by evaporation and the residue was taken up in EtOAc (40 mL). The organic layer was successively washed with a 0.5 M HCl aqueous solution (15 mL), brine (15 mL), a saturated aqueous solution of sodium hydrogen carbonate (15 mL), and brine (15 mL). The solvent was dried over magnesium sulfate and evaporated to dryness. The residue was purified by chromatography (EtOAc/EtOH 80:20). The title compound **5** was obtained as crystals (0.031 g, 39% from **4**). R_f : 0.22 (EtOAc/EtOH 80:20). Mp : 177°C. $[\alpha]_D^{20}$: + 109 (*c* 0.3, CH₃OH), ¹H NMR (300 MHz, CDCl₃) : two rotamers (66:33 ratio), δ = 1.22-1.29 (m, 9H, CH₃), 1.35 (s, 9H, *t*Bu), 1.60, 1.64, 1.67 (s, 3H, CH₃), 1.92-2.12 (m, 3H, H-3), 2.29-2.71 (m, 3H, H-3'), 3.66 (major rotamer) and 3.73 (minor rotamer) (s, 3H, OMe), 3.89-4.42 (m, 9H, H-4, H-4', Hα), 5.47 (d, *J* = 7.6 Hz, NH Boc major rotamer), 6.06 (d, *J* = 8.1 Hz, NH Boc minor rotamer), 7.99 (d, *J* = 7.4 Hz, NH amide major rotamer), 8.26-8.31 (m, 1H, NH amide), 8.63 (d, *J* = 6.3 Hz, NH amide minor rotamer) ppm. ¹³C NMR (75 MHz, CD₃OD): major rotamer δ = 16.3, 16.6, 17.2, 22.0, 22.1, 27.8 (CH₃), 29.0 (*t*Bu), 29.3, 29.7, 29.9 (CH₂), 47.5 (C^α), 47.7 (CH₂), 47.8, 48.3 (C^α), 48.7 (CH₂), 53.1 (CH₃), 69.1, 71.0, 71.1, 80.7 (Cq), 157.9, 173.7, 173.98, 173.9,

174.5, 174.6, 175.1 (CO) ppm. IR (ATR): ν_{\max} 3400, 3359, 2982, 2942, 2891, 1725, 1696, 1657, 1652, 1514, 1425, 1368, 1298, 1246, 1165, 1012 cm^{-1} ; HRMS (ESI, +ve): calculated for $\text{C}_{30}\text{H}_{49}\text{N}_6\text{O}_9$ $[\text{M}+\text{H}]^+$: 637.3561; found 637.3560.

Boc-(S)-Ala-(R)-(α Me)Aze-(S)-Ala-OMe (6)

To a solution of dipeptide **2** (1.28 g, 4.27 mmol) in THF (30 mL) and MeOH (17 mL) was added a solution of sodium hydroxide (0.342 g, 8.55 mmol) in water (3 mL). After stirring at 60°C for 1.5 hour, water (50 mL) was added to the reaction mixture and the organic solvents were evaporated. The remaining aqueous solution was washed with Et_2O (2×10 mL) and acidified to pH = 1 with a 0.5 M HCl aqueous solution (5 mL). The aqueous layer was extracted with CH_2Cl_2 (4×50 mL). The organic layer was dried over magnesium sulfate and evaporated to dryness. The resulting foam (1.029 g, 84%) was dissolved in THF (15 mL) before adding HCl·H-(S)-Ala-OMe (602 mg, 4.31 mmol). To the suspension, cooled to 0°C, HATU (1.64 g, 4.31 mmol) and DIPEA (2.3 mL, 13.32 mmol) were added. After stirring for 72 hours, THF was removed by evaporation and the residue was taken up in CH_2Cl_2 (100 mL). The organic layer was successively washed with a saturated aqueous solution of sodium hydrogen carbonate (50 mL), and brine (25 mL). The solvent was dried over magnesium sulfate and evaporated to dryness. The residue was purified by chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 97:3). The title compound **6** was obtained as crystals (1.046 g, 78% from **2**). R_f : 0.15 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 97:3). Mp: 104°C. $[\alpha]_{\text{D}}^{20}$: +103 (c 1.1, CH_2Cl_2), ^1H NMR (300 MHz, CDCl_3): δ = 1.28 (d, J = 6.9 Hz, 3H, CH_3), 1.39 (d, J = 7.1 Hz, 3H, CH_3), 1.40 (s, 9H, $t\text{Bu}$), 1.78 (s, 3H, CH_3), 2.04-2.13 (m, 1H, H-3), 2.72-2.82 (m, 1H, H-3'), 3.69 (s, 3H, OMe), 3.94-4.03 (m, 1H, H-4), 4.08-4.18 (m, 2H, H-4', H α), 4.43-4.53 (m, 1H, H α), 5.21 (d, J = 9.0 Hz, 1H, NH Boc), 8.41 (d, J = 6.0 Hz, 1H, NH amide) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ = 17.6, 17.8, 22.9 (CH_3), 27.3 (CH_2), 28.3 ($t\text{Bu}$), 45.8 (CH_2), 46.3, 48.2 (C^α), 52.2 (CH_3), 71.3, 79.8 (C_q), 155.1, 172.9, 173.0, 173.2 (CO) ppm. IR (ATR): ν_{\max} 3353, 3309, 3000, 2941, 2879, 1780, 1738, 1687, 1652, 1537, 1448, 1515, 1426, 1367, 1247, 1213, 1165, 1068, 1012 cm^{-1} ; HRMS (ESI, +ve): calculated for $\text{C}_{17}\text{H}_{30}\text{N}_3\text{O}_6$ $[\text{M}+\text{H}]^+$: 372.2135; found 372.2138.

Boc-(R)-(α Me)Aze-OH (7)

To a solution of Boc-(R)-(α Me)Aze-OMe **1** (0.645 g, 2.82 mmol) in THF (20 mL) and MeOH (11 mL) was added a solution of sodium hydroxide (0.225 g, 5.64 mmol) in water (2 mL). After stirring at 20°C for 15 hours, water (50 mL) was added to the reaction mixture and the organic solvents were evaporated. The remaining aqueous solution was washed with Et_2O (4×10 mL) and acidified to pH = 1 with a 0.5 M HCl aqueous solution. The aqueous layer was extracted with CH_2Cl_2 (4×25

mL). The organic layer was dried over magnesium sulfate and evaporated to dryness. The title compound **7** was obtained as a solid (0.617 g, 97% from **1**). R_f : 0.40 (CH₂Cl₂/MeOH 90:10). Mp : 90°C. $[\alpha]_D^{20}$: +115 (*c* 0.5, CH₂Cl₂), ¹H NMR (300 MHz, CDCl₃): δ = 1.48 (s, 9H, *t*Bu), 1.72 (s, 3H, CH₃), 2.02–2.11 (m, 1H, H-3), 2.72–2.81 (m, 1H, H-3'), 3.73–3.91 (m, 2H, H-4, H-4') ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 22.2 (CH₃), 27.6 (CH₂), 28.3 (*t*Bu), 44.5 (CH₂), 69.2 (Cq), 82.6 (Cq), 157.4, 174.7 (CO) ppm. IR (ATR): ν_{\max} 2974, 2894, 2661, 2551, 1722, 1622, 1477, 1452, 1428, 1367, 1195, 1159, 1083, 859, 768, 605 cm⁻¹; HRMS (ESI, -ve): calculated for C₁₀H₁₆NO₄ [M-H]⁻: 214.1079; found 214.1070.

Boc-[(*R*)-(αMe)Aze-(*S*)-Ala]₂-OMe (8**)**

Tripeptide **6** (1.046 g, 2.82 mmol) was dissolved in 1.7 M HCl solution in Et₂O (40 mL). After overnight stirring, a precipitate formed. The solvent was removed under vacuum. To the tripeptide hydrochloride salt THF (28 mL) was added and the mixture was cooled to 0°C. Boc-(*R*)-(αMe)Aze-OH (**7**) (0.606 g, 2.82 mmol), HATU (1.29 g, 3.40 mmol), and DIPEA (3.60 mL, 20.87 mmol) were added. After stirring for 5 days, THF was removed by evaporation and the residue was taken up in CH₂Cl₂ (50 mL). The organic layer was successively washed with a 0.5 M HCl aqueous solution (30 mL), brine (30 mL), and a saturated aqueous solution of sodium hydrogen carbonate (30 mL). The solvent was dried over magnesium sulfate and evaporated to dryness. The residue was purified by chromatography (CH₂Cl₂/MeOH 98.5:1.5). The title compound **8** was obtained as an oil (0.414 g, 88% from **6**). R_f : 0.30 (CH₂Cl₂/MeOH 97:3). $[\alpha]_D^{20}$: +158 (*c* 1.1, CH₂Cl₂), ¹H NMR (300 MHz, CDCl₃): two rotamers (50:50 ratio), δ = 1.29 (d, *J* = 6.7 Hz, 3H, CH₃), 1.34 (d, *J* = 7.3 Hz, 3H, CH₃), 1.39 (s, 9H, *t*Bu), 1.58 (s, 3H, CH₃), 1.67 (s, 3H, CH₃), 1.82-2.01, 2.04-2.13, 2.37-2.80 (m, 4H, H-3 and H-3'), 3.64 (s, 3H, OMe), 3.67-3.69, 3.71-3.86, 3.95-4.03 (m, 3H, H-4 and H-4'), 4.09-4.35 (bs, 2H, H-4' and Hα), 4.37-4.46 (m, 1H, Hα), 7.23 (bs, 0.5H, NH one rotamer), 8.05 (bs, 0.5H, NH one rotamer), 8.25 (bs, 0.5H, NH), 8.34 (bs, 0.5H, NH one rotamer) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 16.6, 17.2, 17.5, 21.8, 22.0, 22.5, 22.8 (CH₃), 27.4, 27.9 (CH₂), 28.3 (*t*Bu), 43.3, 44.5 (CH₂), 45.1, 45.5 (C^α), 45.9 (CH₂), 48.1 (C^α), 52.1 (CH₃), 69.4, 70.6, 71.1, 80.5, 81.0 (Cq), 155.8, 155.1, 171.9, 172.1, 173.0, 173.5, 174.6 (CO) ppm. IR (ATR): ν_{\max} 3337, 2976, 2932, 2890, 1745, 1702, 1655, 1526, 1455, 1430, 1365, 1247, 1154, 1071, 958, 850, 772 cm⁻¹; HRMS (ESI, +ve): calculated for C₂₂H₃₇N₄O₇ [M+H]⁺: 469.2662; found 469.2663.

Boc-[(*S*)-Ala-(*R*)-(αMe)Aze]₂-(*S*)-Ala-OMe (9**)**

Tetrapeptide **8** (0.696 g, 1.48 mmol) was dissolved in 1.7 M HCl solution in Et₂O (12 mL). After overnight stirring, a precipitate formed. The solvent was removed under vacuum. To the

tetrapeptide hydrochloride salt THF (13 mL) was added and the mixture was cooled to 0°C. Boc-(*S*)-Ala-OH (0.336 g, 1.78 mmol), HATU (0.675 g, 1.78 mmol), and DIPEA (1.25 mL, 7.19 mmol) were added. After stirring for 72 hours, THF was removed by evaporation and the residue was taken up in CH₂Cl₂ (80 mL). The organic layer was successively washed with a 0.5 M HCl aqueous solution (20 mL), brine (20 mL), and a saturated aqueous solution of sodium hydrogen carbonate (20 mL). The solvent was dried over magnesium sulfate and evaporated to dryness. The residue was purified by chromatography (CH₂Cl₂/MeOH 97:3). The title compound **9** was obtained as crystals (0.364 g, 45% from **8**). R_f : 0.35 (CH₂Cl₂/MeOH 90:10). Mp : 208°C. $[\alpha]_D^{20}$: +120 (*c* 1.6, CH₂Cl₂), ¹H NMR (300 MHz, CD₃OD): two rotamers (85:15 ratio): major rotamer δ = 1.28 (d, *J* = 5.8 Hz, 3H, CH₃), 1.41-1.47 (bs, 15H, CH₃), 1.70 (s, 3H, CH₃), 1.73 (s, 3H, CH₃), 2.12-2.40 (m, 2H, H-3), 2.43-2.62 (m, 2H, H-3'), 3.75 (s, 3H, OMe), 3.96-4.02 (m, 1H, H_α), 4.07-4.15 (m, 1H, H_α), 4.17-4.52 (m, 5H, H-4, H-4', H_α) ppm. ¹³C NMR (75 MHz, CD₃OD): major rotamer δ = 16.8, 17.2, 18.1, 22.6 (CH₃), 29.6 (*t*Bu), 30.3, 30.6 (CH₂), 48.4, 48.5 (CH₂), 48.6, 48.9, 50.6 (CH), 53.6 (CH₃), 71.5, 71.8, 81.4 (Cq), 158.6, 174.3, 175.3, 175.4, 175.6, 176.0 (CO) ppm. IR (ATR): ν_{max} 3387, 3318, 3042, 2982, 2963, 2938, 2882, 1747, 1687, 1650, 1637, 1540, 1474, 1437, 1304, 1270, 1248, 1178, 1057, 1012, 836, 743 cm⁻¹; HRMS (ESI, +ve): calculated for C₂₅H₄₂N₅O₈ [M+H]⁺: 540.3033; found 540.3038.

2.2 | X-Ray diffraction

Crystals of Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe (**6**) were grown by slow evaporation from a diisopropyl ether solution. X-Ray diffraction data were collected with a Gemini E four-circle kappa diffractometer (Agilent Technologies) equipped with a 92 mm EOS CCD detector, using graphite monochromated Cu K_α radiation (λ = 1.54178 Å). Data collection and reduction were performed with the CrysAlisPro software (Agilent Technologies). A *semi*-empirical absorption correction based on the multi-scan technique using spherical harmonics, implemented in the SCALE3 ABSPACK scaling algorithm, was applied. The structure was solved by *ab initio* procedures of the SIR 2002 program.³⁴ The chosen enantiomorph was based on the known configuration of the constituent α-amino acids. Refinement was carried out by full-matrix least-squares procedures on *F*², using all data, by application of the SHELXL-97 program,³⁵ with anisotropic displacement parameters for all of the non-H atoms. H-Atoms were calculated at idealized positions and refined using a riding model. CCDC 1884933 contains the supplementary crystallographic data for this

paper. These data can be obtained from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Crystal data for Boc-(S)-Ala-(R)-(αMe)Aze-(S)-Ala-OMe (6)

$C_{17}H_{29}N_3O_6$, $M = 371.43$, orthorhombic, space group $P2_12_12_1$, $a = 9.1175(2)$ Å, $b = 9.2378(2)$ Å, $c = 24.8417(6)$ Å, $V = 2092.31(8)$ Å³, $Z = 4$; $\rho_{\text{calcd}} = 1.179$ g cm⁻³, $\mu = 0.744$ mm⁻¹, 15063 reflections measured, 3852 independent reflections ($R_{\text{int}} = 0.0201$), $\theta_{\text{max}} = 69.51^\circ$, $T = 293(2)$ K. The final R_1 values were 0.0523 [$I \geq 2\sigma(I)$] and 0.0560 (all data). The final wR_2 values were 0.1536 [$I \geq 2\sigma(I)$] and 0.1594 (all data). Data / restraints / parameters 3852 / 0 / 235. Goodness of fit on $F^2 = 1.015$. Flack parameter: -0.1(3). The largest difference peak and hole were 0.345 and -0.269 e Å⁻³, respectively.

2.3 | Circular dichroism

The CD spectra were measured on a Jasco model J-715 spectropolarimeter equipped with a Haake thermostat (Thermo Fisher Scientific). Baselines were corrected by subtracting the solvent contribution. Fused quartz cells of 1.0 mm and 10.0 mm path lengths (Hellma) were used. The values are expressed in terms of $[\Theta]_T$, the total molar ellipticity (deg × cm² × dmol⁻¹). Spectrograde TFE (Sigma-Aldrich) was used as solvent.

2.4 | FT-IR absorption

The FT-IR absorption spectra were recorded at 293 K using a Perkin-Elmer model 1720X FT-IR spectrophotometer, nitrogen flushed, equipped with a sample-shuttle device, at 2 cm⁻¹ nominal resolution, averaging 100 scans. Solvent (baseline) spectra were obtained under the same conditions. For spectral elaboration, the software SpectraCalc (Galactic) was employed. Cells with path lengths of 1.0 mm and 10.0 mm (with CaF₂ windows) were used. Spectrograde deuterated chloroform (99.8% *d*) was purchased from Merck.

2.5 | Nuclear magnetic resonance

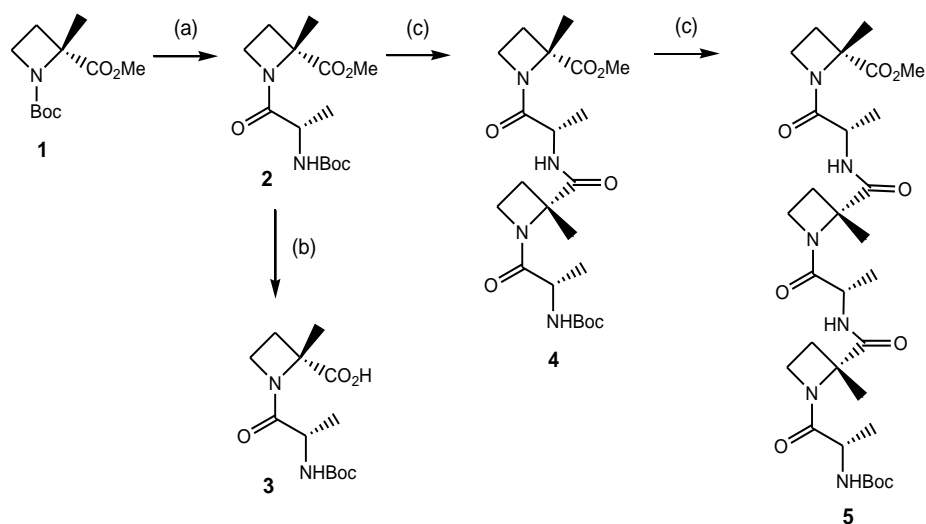
The ¹H NMR experiments were performed on a Bruker AVANCE DMX-600 spectrometer operating at 600 MHz using the TOPSPIN software package. For the signal assignments, the solvent was used as the internal standard: (99.96%, *d*; Acros Organics) CDCl₃ (¹H: $\delta = 7.26$ ppm). The spectra were recorded at 298 K. All homonuclear 2D-spectra were acquired by using 512 experiments, each one consisting of 64–80 scans and 2 K data points. The spin systems of amino

acid residues were identified using standard DQF-COSY³⁶ and CLEAN-TOCSY³⁷ spectra. In the latter case, the spin-lock pulse sequence was 70 ms long.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis

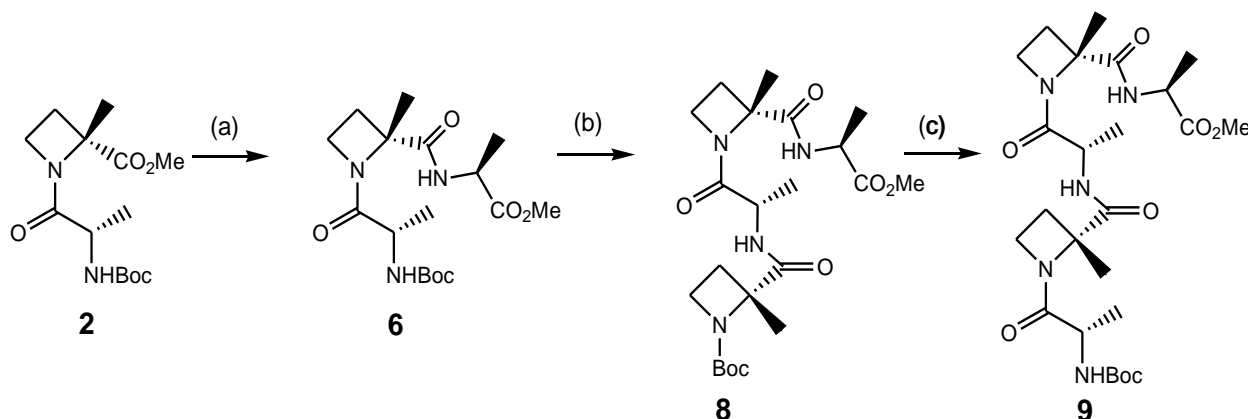
Two series (**A** and **B**) of hetero-chiral sequential peptides based on (*R*)-(α Me)Aze³⁰ and (*S*)-Ala were prepared by chain elongation of N^o-Boc protected peptide segments in DMF solution using HATU as the coupling reagent, in the presence of the tertiary amine DIPEA. The synthesis of series **A** (Scheme 1) began with the Boc cleavage of protected Boc-(*R*)-(α Me)Aze-OMe **1** with HCl in Et₂O, followed by coupling with Boc-(*S*)-Ala-OH to afford the terminally protected dipeptide **2** in 84% yield. Saponification of the methyl ester function of **2** with sodium hydroxide gave the corresponding acid **3** in 81% yield. Boc cleavage of **2** and coupling with Boc-(*S*)-Ala-(*R*)-(α Me)Aze-OH **3** afforded tetrapeptide **4** in 41% yield. The final, fully protected hexapeptide **5** was produced in 39% yield after Boc cleavage of **4** and coupling with Boc-(*S*)-Ala-(*R*)-(α Me)Aze-OH **3**.



Scheme 1 Synthesis of series **A**. (a): i) 1.7 M HCl in Et₂O; ii) Boc-(*S*)-Ala-OH, HATU, DIPEA, DMF (84% two steps). (b): NaOH (81%). (c): i) 1.7 M HCl in Et₂O; ii) dipeptide **3**, HATU, DIPEA, DMF (**4** : 41%, **5**: 39%, two steps).

Similarly, the synthesis of series **B** (Scheme 2) began with the saponification of dipeptide **2** with sodium hydroxide followed by coupling with HCl·H-(*S*)-Ala-OMe to afford the terminally protected tripeptide **6** in 78% yield. Boc cleavage of **6** and coupling with Boc-(*R*)-(α Me)Aze-OH

(7) afforded tetrapeptide **8** in 75% yield. Finally, peptide **9** was produced in 45% yield after Boc cleavage of **8** and coupling with Boc-(*S*)-Ala-OH.



Scheme 2 Synthesis of series **B**. (a): i) NaOH; ii) HCl·H-(*S*)-Ala-OMe, HATU, DIPEA, DMF (78%, two steps). (b): i) 1.7 M HCl in Et₂O; ii) Boc-(*R*)-(αMe)Aze-OH (**7**) (obtained from **1** by saponification with NaOH in 97% yield), HATU, DIPEA, DMF (75%, two steps). (c): i) 1.7 M HCl in Et₂O; ii) Boc-(*S*)-Ala-OH, HATU, DIPEA, DMF (45%, two steps).

3.2 | Crystal-state 3D-structural study

Despite many attempts, among all the terminally protected, hetero-chiral oligopeptides synthesized, we were able to grow single crystals suitable for an X-ray diffraction investigation only for the tripeptide Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe **6** (by slow evaporation from a diisopropyl ether solution). Its molecular structure is shown in Figure 2. Conformationally informative main-chain torsion angles are given in Table 1. The parameters characterizing the intra- and intermolecular H-bonds are listed in Table 2.

Bond lengths and bond angles (deposited as CCDC 1884933) are in good accord with the values already published for the geometry of the Boc-NH urethane group,³⁹ the peptide unit,^{40,41} the methyl ester moiety,⁴² and the (αMe)Aze residue.²⁴

In the (αMe)Aze four-membered azetidine ring, all of the *endocyclic* bond angles are close to 90°, the largest being that at the N atom [94.18(18)°] and the narrowest at C^α [87.79(18)°]. Concerning the *exocyclic* bond angles at N, the value of C'_{*i*-1}-N_{*i*}-C^α_{*i*} is 130.14(17)° and that of C'_{*i*-1}-N_{*i*}-C^γ_{*i*} is 135.4(2)°. As a consequence, the sum of bond angles at N is 359.95(18)°, identical within experimental error to 360°, the value typical of the planar disposition of the bonds at the *sp*²-hybridized nitrogen atom. Moreover, the entire azetidine ring of (*R*)-MeAze is perfectly planar, in

that atomic displacements from its mean plane are all within $\pm 0.002(3)$ Å. The conformationally sensitive N-C $^{\alpha}$ -C' (τ) bond angle⁴²⁻⁴⁵ is 114.34(17)°, remarkably expanded compared to the regular tetrahedral value (109.5°).

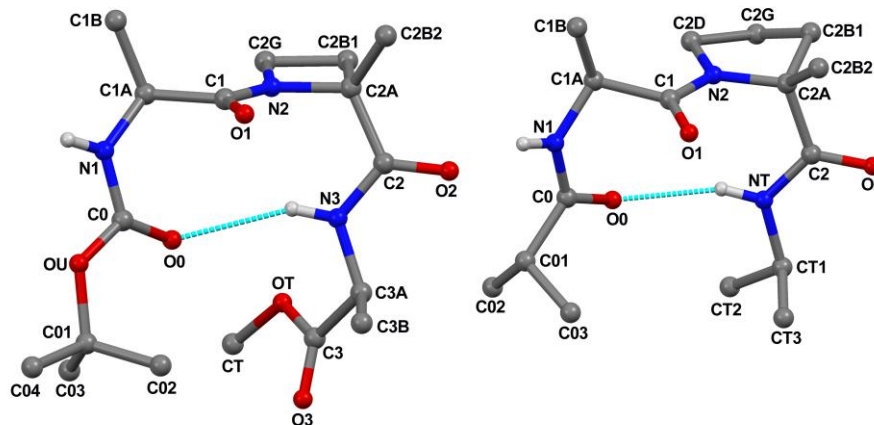


Figure 2 Comparison of the X-ray diffraction structures of the N $^{\alpha}$ -protected tripeptide ester Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe **6** (left, this work) and N $^{\alpha}$ -blocked dipeptide N-alkylamide *i*BuCO-(*S*)-Ala-(*R*)-(αMe)Pro-NHiPr (right, adapted from ref. 48). In each molecule the $i \leftarrow i+3$ intramolecular H-bond is represented as a dashed line.

Table 1 Selected torsion angles (°) for Boc-(*S*)-Ala-(*R*)-MeAze-(*S*)-Ala-OMe **6**

Torsion Angle	
θ_1^a	178.9(2)
ω_0	178.53(19)
ϕ_1	-56.7(3)
ψ_1	139.34(18)
ω_1	-179.37(18)
ϕ_2	72.8(3)
ψ_2	3.8(3)
ω_2	177.5(2)
ϕ_3	-139.9(3)
ψ_3	23.6(4)
ω_3	-176.1(5) ^b

^a C01-OU-C0-N1.

^b C3A-C3-OT-CT.

The urethane, peptide, and ester bonds (ω torsion angles) are observed in the common *trans* planar disposition with deviations from 180° not exceeding $3.9(5)^\circ$. The ϕ, ψ backbone torsion angles are strongly indicative of a type-II β -turn⁵⁻⁷ for the N-terminal hetero-chiral *-(S)-Ala-(R)-*(α Me)Aze- dipeptide sequence [$\phi_1, \psi_1 = -56.7(3)^\circ, 139.34(18)^\circ$; $\phi_2, \psi_2 = 72.8(3)^\circ, 3.8(3)^\circ$]. The C-terminal *(S)-Ala* residue adopts a quite unusual, only partially elongated conformation: $\phi_3, \psi_3 = 139.9(3)^\circ, 23.6(4)^\circ$. This turn structure is stabilized by an $i \leftarrow i+3$ intramolecular H-bond between the peptide N3-H3 group of Ala(3) and the O0 carbonyl oxygen atom of the Boc-urethane moiety. The H-bond parameters are close to those expected^{47,48} for a C=O...H-N intramolecular H-bond. Figure 2 also presents a side-by-side comparison between the X-ray diffraction 3D-structures of Boc-*(S)-Ala-(R)-*(α Me)Aze-*(S)-Ala-OMe* and *i*BuCO-*(S)-Ala-(R)-*(α Me)Pro-NH*i*Pr, the latter published by three of us (M.C., F.F., C.T.) ten years ago.^{38,49} Both compounds are characterized by a strictly similar -CO-*(S)-Ala-(R)-*(α Me)Xxx-NH- N $^\alpha$ -acylated and C-terminally monoalkylamidated, hetero-chiral dipeptide sequence, differing in the chemical structure only by the ring size of the C-terminal, C $^\alpha$ -tetrasubstituted α -amino acid unit: a tetra-atomic ring in (α Me)Aze of the former peptide, with a penta-atomic ring in (α Me)Pro of the latter peptide. Both dipeptide segments are folded in regular, intramolecularly H-bonded, type-II β -turns with the values of their respective ϕ, ψ backbone torsion angles almost overlapping [for the *-(S)-Ala-(R)-*(α Me)Pro- dipeptide sequence they are: $\phi_1, \psi_1 = -54.8(5)^\circ, 133.4(4)^\circ$; $\phi_2, \psi_2 = 78.2(5)^\circ, 0.3(6)^\circ$]. From these data, it is quite clear that the Aze \rightarrow Pro ring size expansion does not produce any significant modification in the overall dipeptide folding.

Table 2 Intra- and intermolecular H-bond and C-H...O contact parameters for Boc-*(S)-Ala-(R)-MeAze-(S)-Ala-OMe* **6**

Donor	Acceptor	Distance (Å)	Distance (Å)	Angle (°)	Symmetry of A
D-H	A	D...A	H...A	D-H...A	
N3-H3	O0	3.106(3)	2.32	153	x, y, z
N1-H1	O2	3.002(3)	2.16	166	$1+x, y, z$
C04-H04B	O3	3.390(5)	2.56	144	$1/2+x, 1/2-y, 1-z$
C1B-H1B2	O1	3.545(4)	2.61	165	$1-x, -1/2+y, 3/2-z$

In the packing mode of Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe **6**, an intermolecular H-bond is observed between the (urethane) N1-H1 group and the (peptide) O2 carbonyl oxygen atom of a (1+x, y, z) symmetry related molecule, thus linking molecules along the *a* direction (Figure 3). Additional contributions to the observed packing mode are provided by C-H...O intermolecular interactions,⁵⁰⁻⁵⁴ of which those with H...O separations less than 2.70 Å are listed in Table 2.

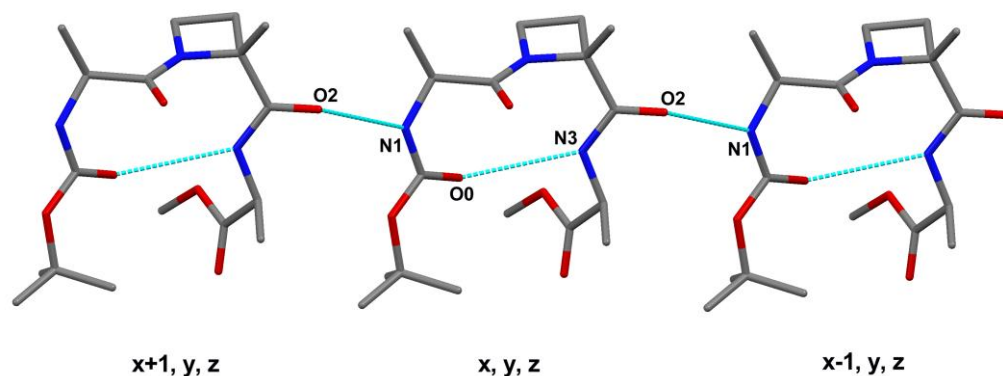


Figure 3 Basic motif of the packing mode in the crystal structure of the tripeptide Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe **6** showing, in addition to the intramolecular O0 ← N3 H-bonds, the intermolecular H-bonds between the N1 and O2 atoms of symmetry related molecules.

3.3 | Conformational analysis in solution

We studied the conformational properties of the terminally protected, hetero-chiral peptides of our - (*S*)-Ala-(*R*)-(αMe)Aze- series in different solvents by the use of electronic CD, FT-IR absorption and NMR spectroscopies.

The CD spectrum of the longest, 3D-structurally most informative oligomer (the hexapeptide **5**) in the polar solvent TFE is reported in Figure 4. It is characterized by two, rather intense, positive Cotton effects at 218 nm and 206 nm, followed by a negative Cotton effect of comparable strength at 188 nm. The cross-over point is seen at 197 nm. The general shape of the CD pattern does not change significantly in other solvents of different polarities (MeOH or acetonitrile, particularly in the latter) (spectra not shown). In MeOH, the slightly diverging relative intensities of the two positive maxima are inverted, the one near 205 nm becoming about 20% stronger than that near 220 nm.

It is clear that this type of CD curve is remarkably different from all those representative of the classical peptide and protein conformations [α-helix, ₃₁₀-helix, β-sheet structure, type-II

poly(Pro)_n helix].^{55,56} On these bases, and considering the short main-chain length of the peptide examined and the number of detailed CD investigations performed on folded sequences adopting β - and γ -turn conformations,⁵⁶⁻⁵⁹ we favor the conclusion that the CD spectrum in Figure 4 is the result of a combination of those arising from an $-(S)\text{-Ala-}(R)\text{-}(\alpha\text{Me})\text{Aze-}$ type-II β -turn conformer (termed class B spectrum, with its strong negative and strong positive amide $\pi \rightarrow \pi^*$ bands near 190 nm and 205 nm, respectively) accompanied by a strong positive amide $n \rightarrow \pi^*$ band near 220 nm associated with a γ -turn conformer centered on $(R)\text{-}(\alpha\text{Me})\text{Aze}$. According to these assignments, the solvent-dependent relative height of the 205 nm and 220 nm bands is attributed to an increase of the type-II β -turn (in MeOH) or of the γ -turn (in either TFE or acetonitrile) population in the solution equilibrium mixture.

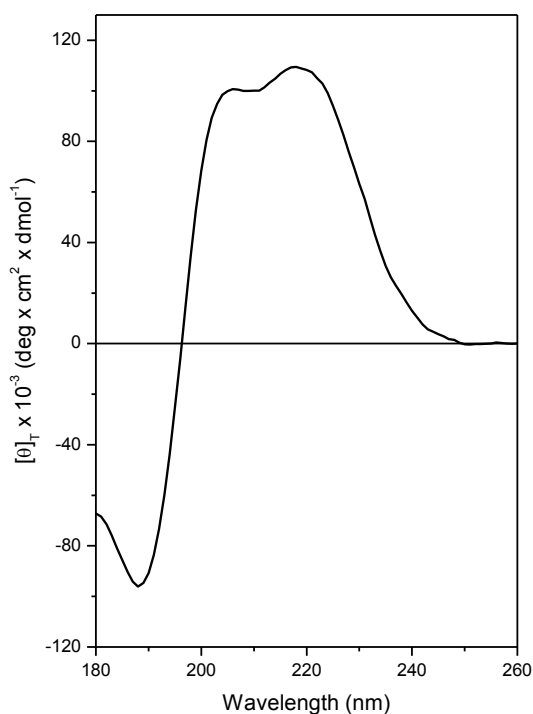


Figure 4 CD spectrum of the hexapeptide Boc-[(*S*)-Ala-(*R*)-(αMe)Aze]₃-OMe **5** in TFE solution. Peptide concentration: 1.0 mM.

Our conformational investigation was expanded to the exploitation of FT-IR absorption spectroscopy, first in the solid state for the tripeptide **6**, the 3D-structure of which we solved by X-ray diffraction (Figure 2), and subsequently on all oligomers in CDCl₃, a solvent of low polarity.

Figure 5 shows the solid-state spectrum of Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe **6** in both the N-H and C=O stretching regions. Positions of the bands are fully consistent with those expected from the intra- and intermolecular H-bonding pattern observed in the crystal state (Figure 3). Specifically, the (Boc-urethane) Ala(1) and (peptide) Ala(3) N-H groups are both H-bonded (bands at 3354 cm⁻¹ and 3315 cm⁻¹), the C-terminal ester C=O group is free (1740 cm⁻¹), the Boc-urethane C=O is H-bonded (1689 cm⁻¹), the -Ala-(αMe)Aze- tertiary amide C=O is free, partially overlapping the band associated with that of the H-bonded C=O of the -(αMe)Aze-Ala secondary amide (1666 cm⁻¹ with shoulder at 1654 cm⁻¹).

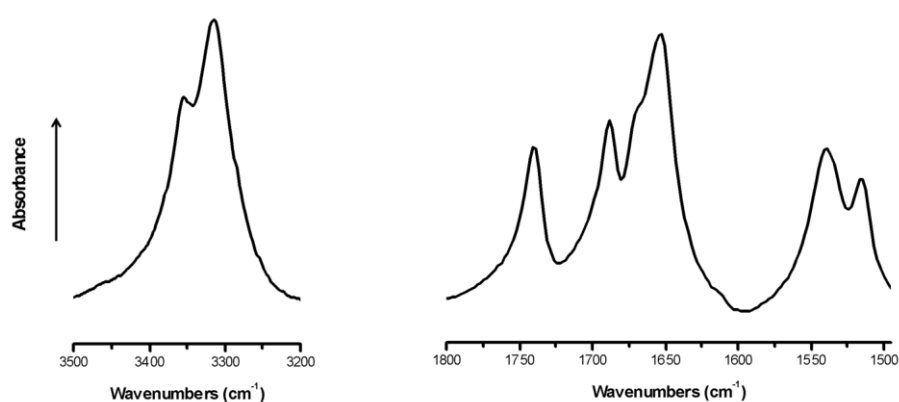


Figure 5 N-H (left) and C=O (right) stretching regions of the FT-IR absorption spectrum of the tripeptide Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe **6** in the solid state.

The FT-IR absorption spectra of four peptides of the -(*S*)-Ala-(*R*)-(αMe)Aze- series, namely dipeptide **2**, tripeptide **6**, tetrapeptide **4** and hexapeptide **5**, at 1.0 mM concentration in CDCl₃ solution are reported in Figure 6. In the N-H stretching region (3500-3200 cm⁻¹), the band at 3430 cm⁻¹ is attributed to free (solvated) N-H groups.⁶⁰ As expected, this is the only band seen in the spectrum of the dipeptide, possessing only one N-H group at the N-terminus which cannot be involved in any folded, intramolecularly H-bonded conformation. Since the intensity of the band(s) below 3400 cm⁻¹ in the spectra of the tripeptide and the higher homologs does (do) not vary significantly upon a tenfold dilution, the absorptions centered near 3350 and 3260 cm⁻¹ should mainly arise from intramolecularly H-bonded groups. In the C=O stretching region (1750-1500 cm⁻¹), band assignments are as follows: at 1742-1738 cm⁻¹ to the C-terminal ester C=O, at 1702 cm⁻¹ to the Boc-urethane C=O, at 1656-1628 cm⁻¹ to the -(αMe)Aze-Ala- secondary and -Ala-(αMe)Aze- tertiary amide C=O groups.⁶¹⁻⁶³

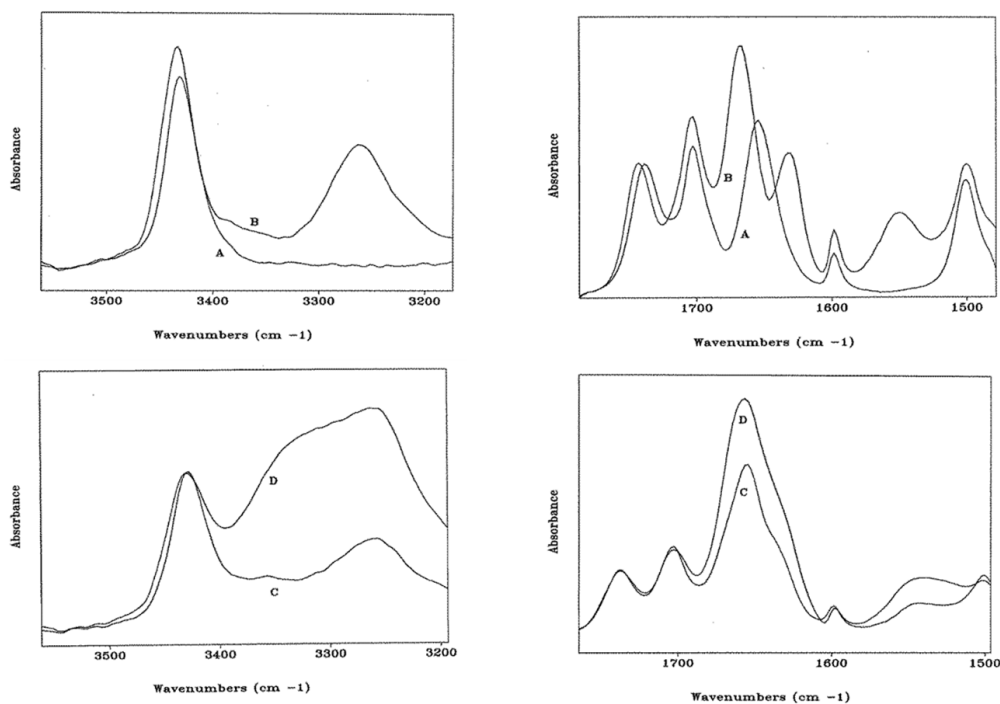


Figure 6 N-H and C=O stretching regions of the FT-IR absorption spectra of the dipeptide **2**, tripeptide **6**, tetrapeptide **4** and hexapeptide **5**, (curves A, B, C and D, respectively) at 1.0 mM concentration in CDCl₃ solution.

A comparison of the spectra of the tripeptide **6** in the solid state (Figure 5) and in solution (Figure 6) strongly suggests that the 3260 cm⁻¹ band (present in solution, but missing in the solid state) is related to an intramolecularly H-bonded γ -turn conformation.^{57,59,61,64} Indeed, the intramolecularly H-bonded N-H group of the β -turn in the solid state must necessarily be associated to one of the two bands at 3354 cm⁻¹ and 3315 cm⁻¹. There is no reason why this band should shift to 3260 cm⁻¹ on going from the solid state to solution. Conversely, in the solution spectrum of the tripeptide, in addition to the 3430 cm⁻¹ (free N-H) and 3260 cm⁻¹ (H-bonded N-H) bands, only a broad and very weak feature is observed at about 3360 cm⁻¹, *i.e.* in the spectral region where a N-H group involved in the β -turn is expected to occur.^{57,61} Further support for the occurrence of a significant population of the γ -turn conformation of the tripeptide in solution is provided by comparison of its C=O stretching region with that of the dipeptide. Both compounds display nearly identical band positions for the ester (1738 cm⁻¹ and 1742 cm⁻¹ in the di- and tripeptide, respectively) and Boc-urethane C=O groups (1702 cm⁻¹ for both peptides). The single, free tertiary amide C=O of the dipeptide vibrates at 1654 cm⁻¹. Conversely, the presence of a secondary and a tertiary amide unit in the tripeptide gives rise to two bands at 1668 cm⁻¹ (more intense) and 1630

cm^{-1} . While the former band is probably due to partially overlapping contributions from both free amides, the position of the 1630 cm^{-1} band is consistent with the involvement of the tertiary amide in an H-bond. The only potential donor of such intramolecular H-bond is the N-H group of Ala(3), thus giving rise to a γ -turn centered on $(\alpha\text{Me})\text{Aze}$.

A literature survey of the available FT-IR absorption solution spectra of peptides characterized by the heterochiral $-\text{CO}-(S)\text{-Ala}-(R)\text{-}(\alpha\text{Me})\text{Xxx-NH-}$ sequence allowed us to facilitate a comparison, although forcibly limited, between those of the $\text{Xxx} = (\alpha\text{Me})\text{Aze}$ tripeptide **6** discussed in this work and the $\text{Ac}-(S)\text{-Ala}-(R)\text{-}(\alpha\text{Me})\text{Pro-NH}i\text{Pr}$ N^q -acylated dipeptide alkylamide reported in De Poli et al.⁴⁹ (the spectrum for the corresponding Aze peptide sequence has not been published to date). Figure 7 shows the overlay of the two curves in the conformationally informative N-H stretching region in CDCl_3 solution. It is evident that below 3400 cm^{-1} , the spectra are quite distinct: that of the sequence $-(S)\text{-Ala}-(R)\text{-}(\alpha\text{Me})\text{Aze-}$ exhibits an intense γ -turn band at 3260 cm^{-1} as mentioned above, whereas a strong β -turn band is apparent at much higher wavenumbers (near 3355 cm^{-1}) for the $-(S)\text{-Ala}-(R)\text{-}(\alpha\text{Me})\text{Pro-}$ sequence. It may be concluded that, at least in CDCl_3 solution, the type of folding propensity shown by the $(\alpha\text{Me})\text{Aze}$ vs. $(\alpha\text{Me})\text{Pro}$ dipeptide motif is remarkably different.

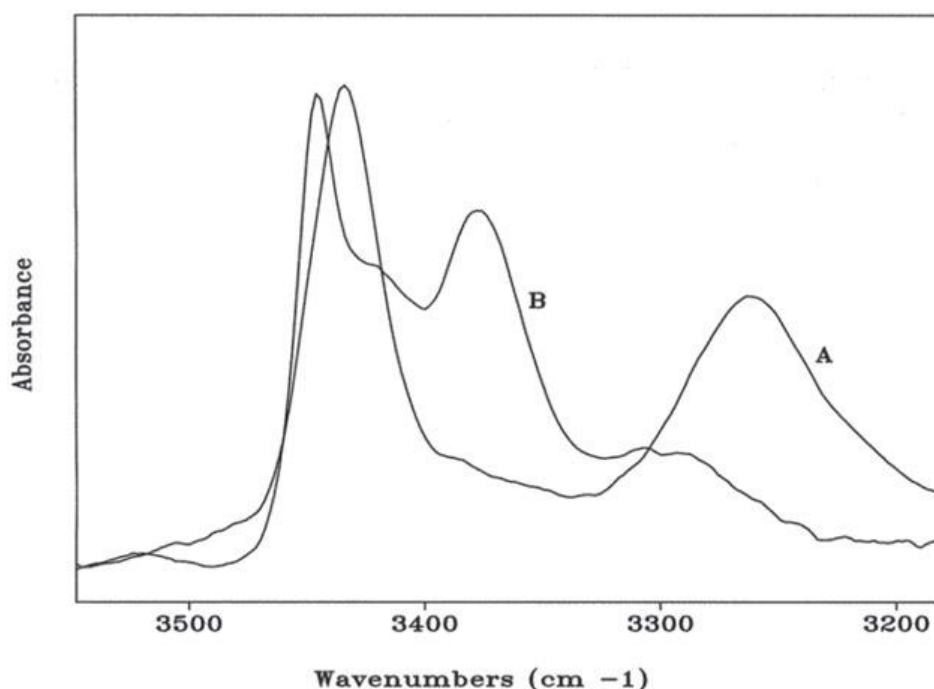


Figure 7 Overlay of the N-H stretching region of the FT-IR absorption spectra of: A, tripeptide **6**, and B, $\text{Ac}-(S)\text{-Ala}-(R)\text{-}(\alpha\text{Me})\text{Pro-NH}i\text{Pr}$ (adapted from De Poli et al.,⁴⁹, Figure S1) at 1.0 mM concentration in CDCl_3 solution

Concerning the higher homologs, a band near 1630 cm^{-1} is also present in the C=O stretching region of the tetra- and hexapeptides as a shoulder of the more intense and broader band

at 1654 cm^{-1} . In the N-H stretching region, as the main-chain length increases from tetra- to hexapeptide, the intensity of the band near 3350 cm^{-1} increases more than that centered at about 3260 cm^{-1} . Overall, the two types of turns seem to coexist in CDCl_3 solution as they do (according to our CD study) in TFE. In addition, the population of the β -turn conformers seems to grow with increasing main-chain length.

More detailed conformational information at the local peptide level was extracted from a 600 MHz ^1H NMR study.

In the 1D-NMR spectrum of the tripeptide Boc-(*S*)-Ala-(*R*)-(α Me)Aze-(*S*)-Ala-OMe **6** in CDCl_3 solution (*Supporting Information*, Figure S1), the Boc-urethane NH proton signal of Ala(1) was unambiguously identified at 5.19 ppm. The Ala(3) NH proton resonates remarkably downfield (at 8.45 ppm), suggesting its involvement in an H-bond. No evidence for signal splitting, potentially arising from *trans-cis* isomerism about the Ala-(α Me)Aze tertiary peptide bond, could be detected in the NMR spectrum of the tripeptide. Conversely, this phenomenon occurs in the NMR spectra of the higher homologs, which are also characterized by extensive signal overlaps, typical of sequential peptides, to the extent that extraction of reliable conformational information from 2D-NMR experiments was precluded. Therefore, a 2D-NMR investigation could be usefully performed on the tripeptide only.

A section of the NOESY⁶⁵ spectrum of the tripeptide Boc-(*S*)-Ala-(*R*)-(α Me)Aze-(*S*)-Ala-OMe **6** in CDCl_3 solution is reported in Figure 8A. The cross-peak between the Ala(3) NH proton and the (*R*)-(α Me)Aze(2) β -CH₃ is much stronger than the cross-peak between the same NH proton and one of the β -CH₂ protons of (*R*)-(α Me)Aze(2). These relative intensities are not consistent with the type-II β -turn conformation found in the crystal state. The related inter-proton distances deduced from the X-ray diffraction structure, 3.76 Å and 3.34 Å (indicated in Figure 8B) do not differ much. Conversely, the relative intensities of the two cross-peaks agree well with the inter-proton distances retrieved from a model of a γ -turn (Figure 8C). Specifically, the distance between the Ala(3) NH proton and the (*R*)-(α Me)Aze(2) β -CH₃, 2.77 Å, is much shorter than the distance between the same NH proton and one of the β -CH₂ protons of (*R*)-(α Me)Aze(2), 4.13 Å. This γ -turn model was obtained starting from the X-ray diffraction structure of the tripeptide but changing the value of the (*R*)-(α Me)Aze(2) ψ torsion angle from 3.8° (see section 3.2 and Table 2) to -45°. This change alone [which obviously disrupts the intramolecular H-bond of the β -turn between the Ala(3) N-H and the Boc-urethane carbonyl oxygen], is sufficient to allow formation of a C=O...H-N H-bond between the Ala(3) N-H and the (tertiary amide) Ala(1) C=O groups. Geometrical

parameters of the newly formed H-bond are: N...O and H...O distances 2.906 Å and 2.11 Å, respectively, and N-H...O angle 155°.

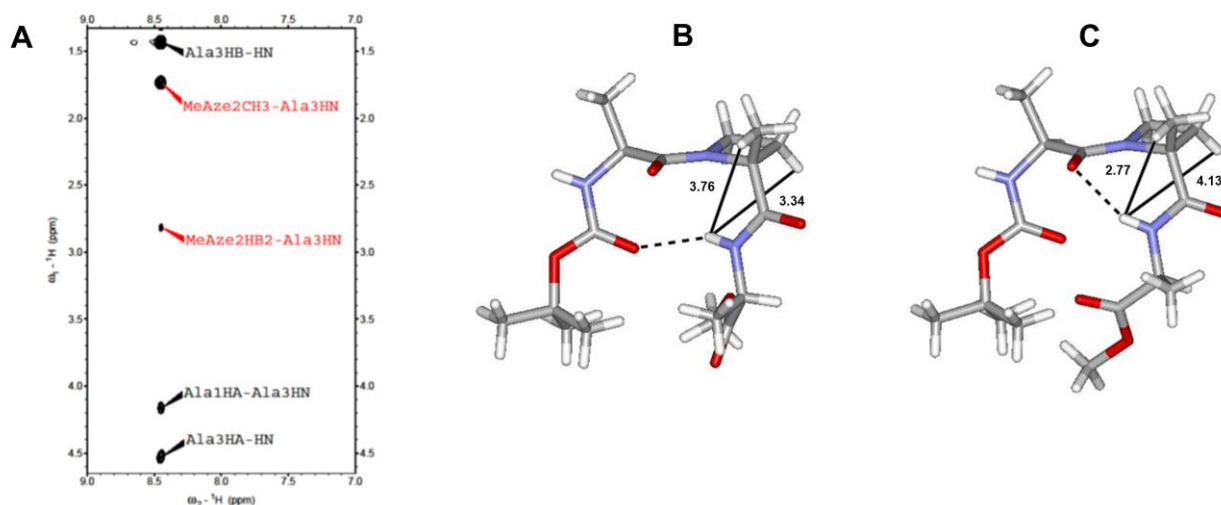


Figure 8 (A) Section of the NOESY spectrum of the tripeptide Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe **6** in CDCl₃ solution. (B) The type-II β-turn conformation found in the crystal state for this compound where the relevant interproton distances are indicated. (C) The relevant interproton distances in the model of the same compound starting from the X-ray diffraction structure and changing arbitrarily the value of the -(*R*)-(αMe)Aze ψ torsion angle from 3.8° (crystal state) to -45°. In **B** and **C**, each intramolecular H-bond is represented as a dashed line.

To summarize, the results from NMR spectroscopy, along with those from CD and FT-IR absorption discussed above, support the view that a γ-turn conformation at the level of the (*R*)-(αMe)Aze residue is remarkably populated in all of the solvents utilized, irrespective of their polarity. A contribution of β-turns seems also to be of relevance in the conformational equilibrium of the longest oligomers.

4 | CONCLUSIONS

In this work, we have synthesized by solution methods a complete set of terminally protected, *hetero*-chiral sequential oligopeptides based on the -(*S*)-Ala-(*R*)-(αMe)Aze- dyad to the hexapeptide level.

Experimental results from our present study support the view that this type of oligopeptide sequence strongly favors folding of the main chain into turns. The occurrence of a type-II β-turn has been unambiguously demonstrated for the tripeptide in the crystal state. However, FT-IR absorption

and 2D-NMR data provide evidence that in CDCl₃ solution the tripeptide is mainly folded in a γ -turn conformation. Concerning its higher homologs, in solution they seem to be involved in conformational equilibria to which both β - and γ -turn conformations may contribute.

It is worth recalling that the γ -turn promoting ability of (α Me)Aze was previously documented by us from the crystal structure of the *homo*-chiral tetrapeptide Boc-[(*S*)-(α Me)Aze-(*S*)-Ala]₂-OMe.²⁴ Moreover, we^{38,49} and others^{57,66} already experimentally demonstrated that the γ -turn region of the ϕ, ψ space, in contrast to (α Me)Aze peptides, was not explored by their (α Me)Pro counterparts. Interestingly, recent theoretical energy evaluations between the two different types of conformations for the (α Me)Aze *vs* (α Me)Pro and Aze mono-peptides (Ac-Xxx-NHMe) strongly support the view that the (α Me)Aze γ -turn is significantly the most stable fold under a number of experimental conditions.³¹ In any case, even if β -turns may prevail over γ -turns in the longest *hetero*-chiral -(*S*)-Ala-(*R*)-(α Me)Aze- oligomers, the resulting overall structure would be a "flat" β -turn ribbon, distinct from the (helical) β -turn ribbon spiral typical of sequential oligopeptides based on the -Aib-Pro- dyad. Finally, the well-known role of any *hetero*-chiral sequence from easily foldable peptides in significantly biasing their overall conformation towards the non-helical type-II β -turn⁴⁻⁷ should also be taken into consideration to fully explain the entire set of our experimental data.

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