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Synthesis and Structural Characterization of 2:1 [α/Aza]-oligomers

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The design of a new β-turn inducer is correlated to the discovery of new foldamers. In this paper, we report the liquid phase synthesis of 2:1 [α/aza]-oligomers using a convergent Boc strategy. The NMR, FTIR, restrained molecular dynamics and X-ray diffraction analyses show that 2:1 [α/aza]-oligomers adopt a C=O(*i*)···H–N(*i* + 2) hydrogen-bonded helical conformation.

Introduction

In recent decades, synthetic and structural studies of new molecules mimicking the secondary structures of peptides and proteins have undergone exponential growth.[1–12] These compounds may be of great therapeutic interest since they are expected to show enhanced resistance to peptidases, better intracellular penetration and improved solubility relative to their natural predecessors. Many studies have focused on the synthesis and structural analysis of pseudopeptidic molecules and a variety of links such as ketomethylenes, retro-inverso, sulfonamides, ethylenes, methyleneamino and many others have been proposed for integration into therapeutically useful agents.[13] In 1998, a new concept of pseudopeptides emerged: foldamers in which a pseudopeptidic modification is repeated regularly throughout the sequence.[14] The term "foldamer" characterizes an oligomer having a strong tendency to adopt a compact and defined conformation that is predictable in solution. The goal is to develop materials that have new and/or improved properties thanks to their well-defined folding behaviour.

For several decades, the "Laboratoire de Chimie-Physique Macromoléculaire" (LCPM), has been involved in the design, synthesis and conformational study of pseudopeptides and pseudopeptidic oligomers.[15–17] In recent years, the LCPM has focused on pseudopeptidic bis-nitrogen compounds. Several studies conducted on hydrazinopeptides[18–20] and *N*-aminopeptides[21–23] have revealed that insertion of an additional nitrogen atom in the peptide backbone results in increased resistance to biodegradation[24] yet also induces the original local structure within these pseudopeptides.[15–17] In this context, we studied a new class of oligomers obtained by oligomerization of an α/aza/α pseudotripeptide. In order to investigate the role of the azaamino acid residue in the oligomer structure and the influence of the absolute configuration of the carbon stereocenters, we decided to study the conformational behavior of homochiral and heterochiral 2:1 [α/aza]-oligomers in solution by using NMR, FTIR and restrained molecular dynamics.

Results and Discussion

Different strategies were explored for the synthesis of aza-peptides featuring combinations of hydrazine and peptide chemistry. The aza-residue was typically constructed from a hydrazine component and a carbonyl-donating reagent.[25–28] A few years ago, we described an original and general method starting from *N*-*tert*-butyloxycarbonylaminophthalimide **1** and involving a Mitsunobu reaction.[29,30] This protocol allowed the synthesis of a series of pseudodipeptides Boc-AzaXaa-AlaOMe (Figure 1).

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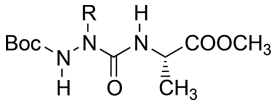
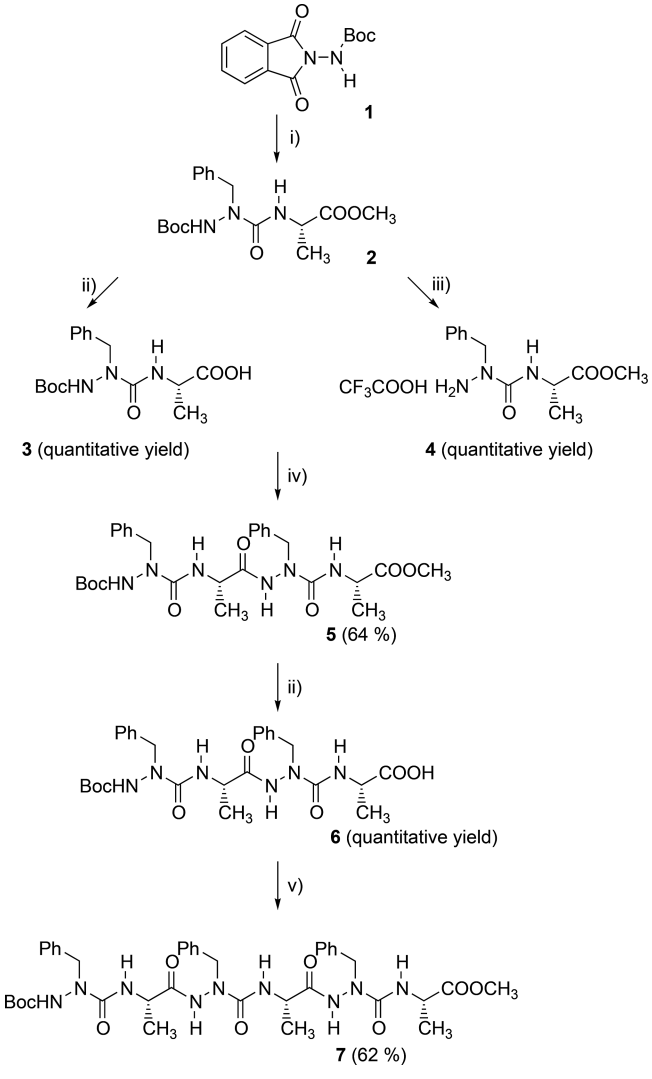


Figure 1. Boc-AzaXaa-AlaOMe [R = CH_3; CH_2Ph; $CH(CH_3)_2$; $CH_2CH(CH_3)_2$; $(CH_2)_2SCH_3$; $(CH_2)_4NHZ$; CH_2COOCH_2Ph].

In this paper, we describe the synthesis and conformation analysis of oligomers containing aza-α units.

In a first approach, 1:1 [α/aza]-oligomers, exhibiting an alternating aza-phenylalanine to (L)-alanine structure, were successfully obtained by oligomerization of the azaPhe-Ala pseudodipeptide using a classical peptide coupling protocol (Scheme 1). Saponification of the ester group of **2** afforded corresponding free *C*-terminal compound **3**. On the other hand, removal of the Boc protection within **2** led to corresponding free *N*-terminal compound **4**.

Homochiral compounds **5** and **7** were obtained in satisfactory yields by coupling respectively, compound **3** with compound **4** and the free *N*-terminal pseudodipeptide **4** with the free *C*-terminal of **6** using HATU/NMM coupling methodology.[31]



Scheme 1. Synthesis of 1:1 [α/aza]-oligomers **5**, **6**, **7** from *N*-*tert*-butyloxycarbonylaminophthalimide (**1**). i) Ref.[29]; ii) NaOH 1 M, CH_3CN; iii) CF_3CO_2H, CH_2Cl_2; iv) HATU, NMM, CH_2Cl_2; v) HATU, NMM, **4**, CH_2Cl_2.

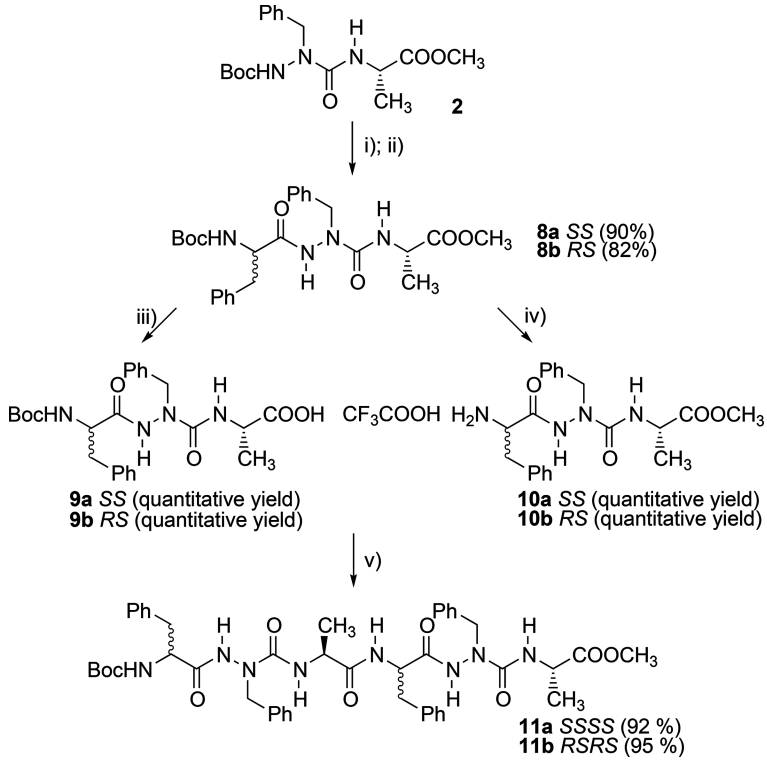
Unfortunately, it was not possible to collect secondary structure information either in the solid state or in solution from this series because of the presence of too many conformers. Since the repetition of an aza-α sequence was not sufficient to induce a regular fold in the molecule, we then decided to study the oligomerization of an α/aza/α-sequence in order to generate 2:1 [α/aza]-oligomers. These new oligomers contained the same aza-α sequence as **5** and **7**. However in 2:1 [α/aza]-oligomers two aza-α sequences are separated by one natural amino acid.

Two diastereoisomeric series of oligomers were synthesized in order to evaluate and compare the impact of carbon stereocenter absolute configurations on overall oligomer conformations. Central to this objective, **8a** and **8b** were obtained by coupling the free *N*-terminus of dipeptide **2** with either (L) or (D) Boc-Phe-OH (Scheme 2). Best yields were obtained when using the fluoride acid coupling method.[32,33] Saponification of the ester group of **8a** and **8b** led respectively to the corresponding free *C*-terminal compounds **9a** and **9b**. On the other hand, removal of the Boc groups on **8a** and **8b** afforded the corresponding free *N*-terminal compounds **10a** and **10b**, respectively

As only natural amino acids residues are involved in the coupling process, homochiral and heterochiral compounds **11a** and **11b** were obtained in very good yields using classical coupling reactions between **9a** and **10a** and **9b** and **10b**, respectively. Homochiral **8a** gave good-quality crystals enabling us to determine its structure in the solid state (Figure 2).[29]

X-ray diffraction analysis shows that **8a** is folded by an *i* + 3→*i* hydrogen bond involving the (Ala)NH and (Boc) CO groups. As a consequence, a pyramidal geometry of the α-nitrogen is observed leading to an R absolute configuration of the aza residue (see Figure S20 in Supporting Information). All the amide bonds are *trans*-planar and the values of the torsional angles are typical of a β_{II}-turn. This fold has already been observed by our group for the Pro-AzaXaa-OH pseudopeptide (with Xaa = Ala or Asn) and was attributed early on to the presence of the proline.[16] Our new data obtained on pseudopeptide **8a** are highly interesting as they show that the introduction of an aza modification induces a turn *even in the absence of a proline residue*. This result is in agreement with the NMR studies and ab initio calculations described by Lee et al.[34,35]

IR and NMR studies were then performed to get information concerning the conformational behaviour of **8a** and **8b** in solution. FT-IR spectroscopy provides evidence of intramolecular hydrogen bond in solution. Attention was focused on the most informative frequency domains corresponding to the NH (3200–3500 cm^{-1}) and CO (1550–1750 cm^{-1}) stretching vibrations. A free secondary amide group was expected to give rise to a NH absorption band located at 3400–3450 cm^{-1} and a CO band at 1650–1700 cm^{-1}. When involved in a classical hydrogen bond, these absorptions are shifted to lower frequencies.[36]



Scheme 2. Synthesis of 2:1 [α/aza]-oligomers **8**–**11** from aza/α-dipeptide **2**. i) CF_3CO_2H, CH_2Cl_2; ii) $C_3F_3N_3$, BocPheOH, pyridine, CH_2Cl_2; iii) NaOH 1M, CH_3CN; iv) CF_3CO_2H, CH_2Cl_2; v) HBTU, DIEA, CH_2Cl_2/DMF.

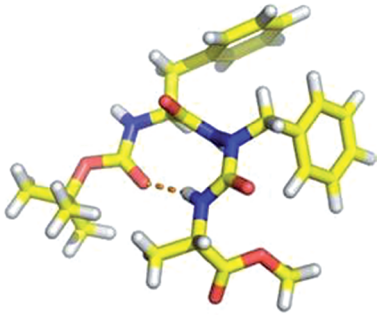


Figure 2. Crystal structure of Boc-Phe-azaPhe-Ala-OMe **8a**. The β-turn is stabilized by hydrogen bonding between the amide of the alanine residue and the carbonyl of Boc protecting group (orange dots).

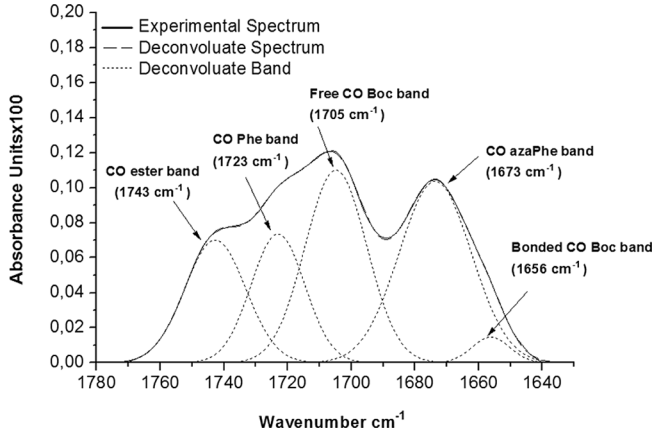


Figure 3. IR spectrum: deconvolution of CO vibrations in **8a**.

First, a very visible band located at 3370 cm^{-1} can be assigned to the NH stretching vibration of the hydrogen-bonded (Ala)NH amide proton of **8a** and **8b**. Thanks to application of second derivatives technique, we were able to assign all CO stretching vibrations of compounds **8a** and **8b** especially a small band at 1655 cm^{-1}, which was assigned to a bonded CO of the Boc group (Figure 3).

^{1}H NMR experiments were carried out using various ratios of $CDCl_3$ and [D_6]DMSO in order to detect NH protons involved in hydrogen bonding.[24] Solvents with hydrogen-bond acceptor atoms, such as [D_6]DMSO, are able to form intermolecular H-bonds producing a downfield displacement for free acidic protons, which is less significant when protons are involved in an intramolecular H-bond.

A variation of less than 1 ppm (0.7 ppm for **8a** and 0.4 ppm for **8b**: see Figures S13 and S14 in Supporting Information) was observed for the NH proton of the alanine residue of both compounds **8a** and **8b**. These low values, compared to the shifts observed for the other NH protons (more than 2 ppm), attest to the fact that these protons are involved in hydrogen bonding in both the homochiral and heterochiral series. Finally, molecular modelling experiments based on NMR structural constraints (Figure 4) confirmed the possibility of hydrogen bonding between the NH

of alanine residue and the Boc CO group. Structures were refined using molecular dynamic simulations on a 1 ns timescale in explicit solvent (a chloroform box). The structures clearly reveal the presence of a β-turn and close analysis of the dihedral angles enables their classification as a type IV β-turn. The folding of the main peptide chain implies that the phenylalanine NH group is directed to the outside of the molecular center and therefore cannot be involved in a hydrogen bonding.

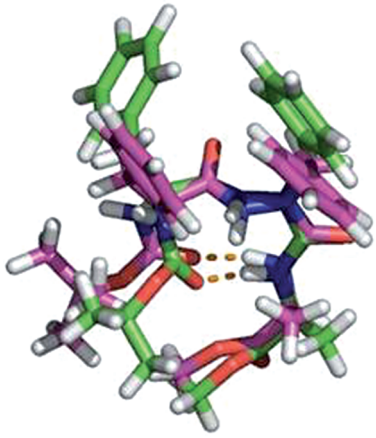


Figure 4. Overlay of the NMR structure in $CDCl_3$ at 308 K of the **8a** (green) and **8b** (pink). Orange dots represent hydrogen bonds that stabilized the β-turn in **8a** and **8b** respectively.

Both oligomers **8a** and **8b** exhibit a β-turn fold stabilized by a hydrogen bond between the amidic proton of the alanine and the carbonyl oxygen of the Boc protecting group. The structures may be superimposed with a root mean square deviation (rmsd) on the position of backbone atoms (excluding Boc and OMe protecting groups) of 0.29 Å. This is an acceptable result considering the size of the pseudopeptide and the fact that the αN atom of the azaPhe residue can adopt different configurations. This result is in agreement with the β-turn previously reported in the literature for an α-aza-α sequence.[37,38]

We were not able to perform these kinds of experiments for **11a** and **11b**. Analysis of these oligomers required that NMR experiments be carried out below 290 K, a temperature for which DMSO is in a solid state.

Crystallization of homochiral (*S*,*S*,*S*,*S*) compound **11a** gave good-quality crystals that enabled structure determination using X-ray diffraction analyses (Figure 5).

In the crystal structure of **11a** two turns are observed (Figure 5, a). The first is a type I turn stabilized by hydrogen bonding between the carbonyl of phenylalanine 1 and the amide proton of phenylalanine 4. The second turn, a β_{II}-turn, is similar to the turn observed in the crystal structure of **8a**.

Experimental NMR spectroscopic data were collected in solution on oligomers **11a** and **11b** at 253 K and 308 K (see Figure S15 in the Supporting Information). Some constraints can be observed at both temperatures. However, structural constraints were collected at 253 K where the mobility of the oligomers was reduced.

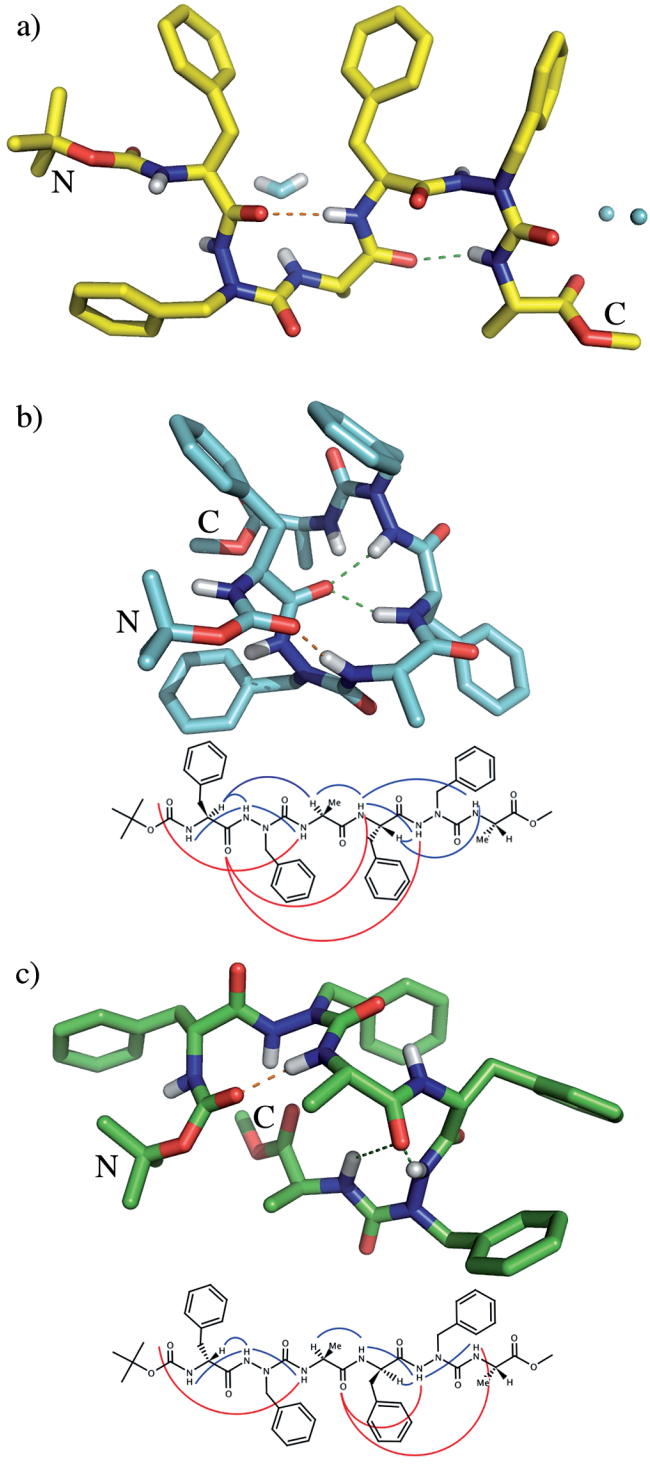


Figure 5. Representation of the main conformation adopted by: a) **11a** in solid state (X-ray data) in yellow; b) **11a** in $CDCl_3$ at 253 K solution (Molecular modelling calculations based on NMR structural restraints) in blue; c) **11b** in $CDCl_3$ solution at 253 K (Molecular modelling calculations based on NMR structural restraints) in green. Water molecules are represented in cyan and hydrogen bonds related to *N*-terminal turns are represented with orange dots. Hydrogen bonds related to *C*-terminal turns are represented in green. *N*- and *C*-terminal extremities are represented by N and C letters, respectively. Hydrogen atoms have been omitted for clarity. In b) and c) schemes represent the NOE constrains used for calculations (in blue) and hydrogen bonds (in red).

Both **11a** and **11b** oligomers exhibit at the *N*-terminal end, a type IV β-turn stabilized by a hydrogen bond between the carbonyl of the Boc protecting group and the amidic proton of alanine 3; this association is similar to the turn observed in the solution and crystal structures of **8a** (see Figures S18 and S19 in Supporting Information). In the *C*-terminal part, the oligomers have different structures. Oligomer **11a** displays a turn stabilized by hydrogen bonding between the carbonyl of phenylalanine 1 and the NH proton of the phenylalanine 4 or aza-phenylalanine 5 to form C10 or C14 pseudocycles, respectively (Figure 5, b). The *C*-terminal turn of **11b** is characterized by C7 or C10 pseudocycles stabilized by hydrogen bonds between the carbonyl of alanine 3 and NH proton of aza-phenylalanine 5 or alanine 6 respectively (Figure 5, c).

The major difference observed between NMR and X-ray diffraction structures lies in the orientations of the *C*-terminal part of the molecule. In the solid state, the molecule assumes a globally extended conformation whereas the NMR spectroscopic data obtained in $CDCl_3$ indicate that both *N*- and *C*-ends are very close to each other (Figure 5, b, c). This difference can be explained by the presence of three water molecules that co-crystallized with **11a**. Two of these form a solvent channel in the crystal and are relatively disordered. In contrast, the third water molecule is trapped between two oligomers and forms three hydrogen bonds with them. We believe that the water molecules might be responsible for the unfolded structure in the solid state by inducing electrostatic repulsions. As a result, X-ray diffraction experiments show that **11a** was structured as a series of two β-turns describing a loose helix (Figure 5, a). On the contrary, the structure of **11a** calculated from NMR distance constraints (Figure 5, b) shows that the solution state facilitates approximate co-localization of the *N*- and *C*-terminal extremities.

Conformational differences between the neat state and solution state have already been reported in the literature by Weiss and Adams.[39,40]

Crystals of heterochiral **11b** failed to meet the minimum requirements necessary for successful X-ray diffraction studies.

Comparisons of NMR spectroscopic data for **11a** and **11b** revealed that the **11a** oligomer can adopt at least three different conformations (Figure 6, A) whereas **11b** is present in only one conformation. (Figure 6, B). Similar spectral data obtained for **11a** in acetone (solvent of crystallization) supports these observations (see Figure S21 in the Supporting Information). It is interesting to notice that the NH proton of aza-phenylalanine residue azaF5 of **11a** is more deshielded than that of oligomer **11b**. This observation suggests that the corresponding hydrogen bond is stronger in **11a**, which also supports the observed reduction in mobility of the oligomer.

Furthermore, molecular dynamic calculations carried out on the basis of solution NMR distance constraints show that both **11a** and **11b** oligomers adopt an almost closed cyclic shape. This structure was confirmed by ROE correlations between Boc group methyls and OMe protecting groups in both oligomers. Moreover, the ROE correlation observed between *N*- and *C*-terminal ends in **11a** suggests that the extended X-ray diffraction structure obtained for this oligomer is probably a minor conformation in solution. However, after calculation, several conformers were observed and the backbones found to oscillate between β- and γ-turns; this is likely due to steric hindrance issues involving the two terminal groups.

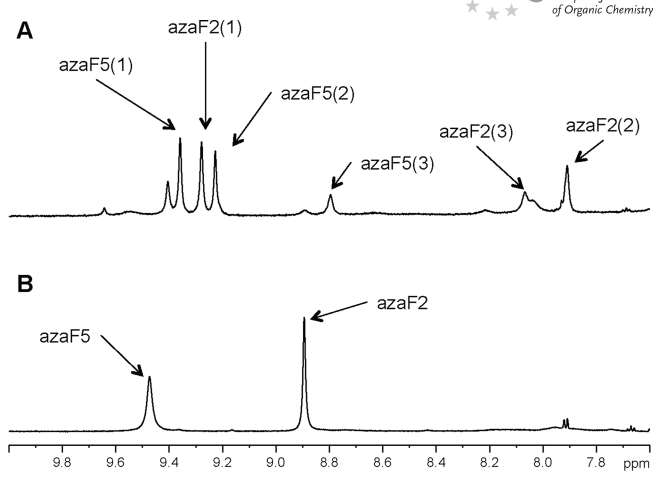


Figure 6. 1D NMR spectra ($CDCl_3$, 253 K) of **11a** (A) and **11b** (B) oligomers. The NH proton of azaPhe residue (azaF) are reported.

Conclusions

Conformational analyses of 2:1 [α/aza]-oligomers were performed to understand the impact of aza-amino acids within peptidic chains. X-ray data, NMR and FT-IR experiments demonstrated that 2:1 [α/aza]-oligomers adopt a C=O(*i*)···H–N(*i* + 2) hydrogen-bonded helical conformation. We also investigated the influence of the chirality of neighboring amino acids. An alternating pattern of *R*,*S* for the α carbons in **11b** appears to reduce the mobility and promote one major conformation whereas several conformations were observed in solution for homochiral oligomer **11a**. The structure stabilized in the solid state and observed in crystal structure assumes a loose helix conformation, one of several possibilities. However, this is a minor form since none of the solution structures determined on the basis of solution state NMR distance constraints duplicates this result. Finally, the solution state structure facilitates neighboring of the *N*- and *C*-terminal ends thus likely facilitating oligomer cyclization. This will be the subject of a forthcoming study.

Experimental Section

General: Starting materials were purchased from Aldrich, Acros Organics, Merck, Fluka, Senn Chemicals, Novabiochem, etc. and used without any purification. THF was dried and distilled from sodium and benzophenone, dichloromethane over P_2O_5 or $LiAlH_4$. The reactions were monitored by Thin Layer Chromatography

(TLC) using Kieselgel 60 with fluorescent indicator UV_{254} (purchased from Merck or Macherey–Nagel). Detection was performed by UV or phosphomolybdic acid. Chromatographic columns were made using silica gel 60 (70–200 μm). All yields have been calculated from pure isolated products. NMR spectra were recorded with a BRUKER AVANCE spectrometer operating at 300 MHz (300 K, for the characterization of compounds) or 600 MHz (253 K or 308 K, for the structural sutdies), in deuterated chloroform ($CDCl_3$) or deuterated acetonitrile (CD_3CN). Chemical shifts are given using tetramethylsilane (TMS) as an internal standard (δ = 0 ppm for TMS). Infrared spectra were recorded with a Bruker TENSOR 27 spectrometer. All IR spectra (64 scans) were obtained at 2 cm^{-1} resolution using a 500 μM CaF_2 solution cell and a dry air purged Bruker Tensor 27 equipped with liquid nitrogen cooled MCT-detector. All samples were dissolved in spectrophotometric grade chloroform (≥ 99.8%, Sigma–Aldrich). All spectra were baseline corrected and smoothed with the Savitzky–Golay algorithm. Electron spray ionization mass spectra (ESI-MS) were recorded using a Bruker MicroTof-Q HR spectrometer.

Representative Procedure for *N*-Terminal Deprotection with TFA: To a stirred solution of Boc protected compound (1 equiv.) in CH_2Cl_2, CF_3COOH (10 equiv.) was added at 0 °C. The resulting solution was stirred until completion monitored by TLC (4 h to 12 h) and concentrated under vacuum. The CF_3COOH was co-evaporated with toluene (3 times), Et_2O (twice) and CH_2Cl_2. The residue was used in subsequent coupling reactions without further purification.

Representative Procedure for *C*-Terminal Deprotection with NaOH 1 M/CH_3CN: To a stirred solution of methyl ester protected compound (1 equiv.) in CH_3CN, an aqueous solution of NaOH 1M (2 equiv.) was added at 0 °C. The resulting solution was stirred until completion monitored by TLC (4 h to 12 h) and aqueous HCl 2 M was added under vigorously stirring (pH = 2). Then CH_2Cl_2 was added and aqueous layer was extracted twice with CH_2Cl_2. Combined organic layers were dried with $MgSO_4$ and the solvents evaporated. The residue was used in subsequent coupling reactions without further purification. Importantly, sometimes a little amount of MeOH is necessary to dissolve the starting compound and/or to minimize emulsification during the extraction.

Representative Procedure for Coupling Reaction with HATU/NMM: To a stirred solution of the CF_3COOH, amine partner (1 equiv.) in CH_2Cl_2 were successively added at room temp. NMM (3 equiv.), acid partner (1 equiv.) and HATU (1 equiv.). After a night to 24 h, the mixture was diluted with aqueous HCl 1N under vigorously stirring (pH = 2). The organic layer was washed with water, aqueous $NaHCO_3$ 0.5N, brine, dried with $MgSO_4$ and the solvents evaporated.

Boc-(azaPhe-Ala)$_2$-OMe (5): Purified by flash chromatography (petroleum ether/EtOAc = 70:30), yield 64%, white powder, m.p. 160–161 °C. IR (ATR): $\tilde{\nu}_{max}$ = 3212, 3326 (NH), 1743, 1682, 1641 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.32–1.40 (m, 6 H, 2×βCH$_3$ Ala), 1.40 (s, 9 H, Boc), 3.68 (s, 3 H, OCH_3), 4.06 (br. s, 1 H, αCH Ala), 4.44 (m, 1 H, αCH Ala), 5.10 (br. s, 4 H, N^{α}-Bn), 5.65 (br. s, 1 H, NHBoc), 6.18 and 6.38 (br. s, 1 H, NH), 7.18–7.34 (m, 10 H, Ar), 8.17 (br. s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.4 (CH_3, Ala), 18.4 (CH_3, Ala), 28.7 (3 CH_3, Boc), 50.2 (CH, Ala), 51.5 and 51.8 (CH_2, N^{α}-Bn), 52.9 (O-*C*H$_3$), 83.1 (C, Boc), 128.2 (CH, Ar), 128.8 (CH, Ar), 129.2 (CH, Ar), 129.5 (CH, Ar), 129.6 (CH, Ar), 136.5 (C, Ar), 137.5 (C, Ar), 154.9 (C=O, Boc), 157.8 (O=C-NH), 158.7 (O=C-NH), 172.3 (O=C-NH), 174.9 (C=O, ester) ppm. HRMS (ESI) calculated for $C_{28}H_{38}N_6NaO_7$ $[M + Na]^+$ *m/z* 593.2694, found 593.2701.

Boc-(azaPhe-Ala)$_3$-OMe (7): Purified by flash chromatography (petroleum ether/EtOAc = 80:20), yield 62%, white powder, m.p. 187–188 °C. IR (ATR): $\tilde{\nu}_{max}$ = 3326, 3212 (NH), 1749, 1737, 1701, 1689, 1678, 1663, 1655, 1637, 1626 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.24–1.41 (m, 12 H, βCH$_3$ Ala and Boc), 3.70 (s, 3 H, OCH_3), 3.99–4.48 (m, 3 H, 3×αCH Ala), 5.11–5.69 (m, 6 H, CH_2, 3×N^{α}-Bn), 6.33, 6.41 and 6.66 (m, 3 H, 3×NH), 7.18–7.37 (m, 15 H, Ar), 8.59 and 8.75 (m, 3 H, 3×NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.5 and 18.6 (CH_3, Ala), 28.6 (3 CH_3, Boc), 50.2 (CH, Ala), 51.7 and 52.6 (CH_2, N^{α}-Bn), 52.7 (O-*C*H$_3$), 127.7 (CH, Ar), 128.3 (CH, Ar), 129.0 (CH, Ar), 129.2 (CH, Ar), 129.5 (CH, Ar), 129.6 (CH, Ar), 135.5 (C, Ar), 137.1 (C, Ar), 137.7 (C, Ar), 154.9 (C=O, Boc), 158.0 (O=C-NH), 159.1 (O=C-NH), 159.4 (O=C-NH), 172.8 (C=O, ester) ppm. HRMS (ESI) calculated for $C_{39}H_{51}N_9NaO_9$ $[M + Na]^+$ *m/z* 812.3702, found 812.3677.

Representative Procedure for the Preparation of Acid Fluoride: To a stirred solution of Boc-Phe-OH (1.5 equiv.) in dry CH_2Cl_2 and pyridine (1.5 equiv.), kept under a N_2 atmosphere, was slowly dropwise added cyanuric fluoride (3 equiv.) at –20 °C. The solution was stirred at –10 °C during 1 or 2 h and a precipitate or emulsion formed and gradually increased amount. Crushed ice was added along with additional CH_2Cl_2. The organic layer was separated and the aqueous layer extracted with cold CH_2Cl_2. The combined organic layers were washed with ice-cold water, dried with $MgSO_4$ and the solvent was removed with a rotary evaporator at room temperature.

Representative Procedure for the Coupling Reaction: Boc-Phe-F (1.5 equiv.) in CH_2Cl_2 was added dropwise to a stirred solution of CF_3COOH, H-azaXaa-Ala-OMe (1 equiv.) in a heterogeneous medium of CH_2Cl_2 and $NaHCO_3$ (2 equiv.) in H_2O (pH = 7). The mixture was stirred overnight at room temperature. After washing of the CH_2Cl_2 solution twice with HCl 1M, $NaHCO_3$ 1M and saturated NaCl, the crude product was dried with $MgSO_4$ and the solvent was removed under vacuum.

Boc-Phe-azaPhe-Ala-OMe (8a): Purified by flash chromatography (Petroleum ether/AcOEt = 50:50), yield 90%, white powder, m.p. 150 °C. IR (ATR): $\tilde{\nu}_{max}$ = 3439, 3375 (NH), 1783, 1743, 1735, 1703 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.37–1.45 (m, 12 H, βCH$_3$ Ala and Boc), 2.82–3.13 (m, 2 H, βCH$_2$ Phe), 3.71 (s, 3 H, OCH_3), 4.02 (m, 1 H, αCH Phe), 4.28 (br. s, 1 H, NHBoc), 4.45–4.50 (m, 1 H, αCH Ala), 4.88–4.95 (m, 2 H, CH_2, N^{α}-Bn), 6.18 (br. s, 1 H, NH Ala), 7.06–7.30 (m, 10 H, Ar), 7.68 (br. s, 1 H, NH azaPhe) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.7 (CH_3 Ala), 28.9 (3 CH_3 Boc), 37.9 (CH_2 Phe), 50.1 (CH Ala), 52.0 (CH_2, N^{α}-Bn), 52.9 (O-CH_3), 56.0 (CH Phe), 81.6 (C, Boc), 128.1 (CH, Ar), 128.3 (CH, Ar), 129.2 (CH, Ar), 129.4 (CH, Ar), 129.7 (CH, Ar), 129.9 (CH, Ar), 136.5 (C, Ar), 137.1 (C Ar), 156.5 (C=O, Boc), 157.6 (O=C-NH), 171.1 (O=C-NH), 174.9 (C=O, ester) ppm. HRMS (ESI) calculated for $C_{26}H_{34}N_4NaO_6$ $[M + Na]^+$ *m/z* 521.2371, found 521.2344.

Boc-D-Phe-azaPhe-Ala-OMe (8b): Purified by flash chromatography (Petroleum ether/AcOEt = 50:50), yield 82%, white powder, m.p. 72 °C. IR ($CDCl_3$): $\tilde{\nu}_{max}$ = 3439, 3372 (NH), 1741, 1721, 1705, 1674 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 9 H, Boc) 1.39 (d, *J* = 7.3 Hz, 3 H, βCH$_3$ Ala), 2.82–3.13 (m, 2 H, βCH$_2$ Phe), 3.70 (s, 3 H, OCH_3), 4.11 (m, 1 H, αCH Phe), 4.42–4.47 (m, 1 H, αCH Ala), 4.65 (m, 2 H, CH_2, N^{α}-Bn), 4.78 (br. s, 1 H, NHBoc), 6.05 (br. s, 1 H, NH Ala), 7.12–7.33 (m, 10 H, Ar), 7.53 (br. s, 1 H, NH azaPhe) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.5 (CH_3 Ala), 28.9 (3 CH_3 Boc), 37.9 (CH_2 Phe), 49.3 (CH Ala), 50.0 (CH_2, N^{α}-Bn), 52.8 (O-CH_3), 56.2 (CH Phe), 81.9 (C, Boc), 127.5 (CH, Ar), 128.1 (CH, Ar), 128.3 (CH, Ar), 129.2 (CH, Ar),

129.5 (CH, Ar), 129.7 (CH, Ar), 129.8 (CH, Ar), 136.3 (C, Ar), 137.1 (C Ar), 156.7 (C=O, Boc), 157.5 (O=C-NH), 171.1 (O=C-NH), 174.7 (C=O, ester) ppm. HRMS (ESI) calculated for $C_{26}H_{34}N_4NaO_6$ $[M + Na]^+$ *m/z* 521.2371, found 521.2389.

Representative Procedure for the Coupling Reaction with HBTU/DIEA: To a stirred solution of the CF_3COOH-amine partner salt (1 equiv.) in CH_2Cl_2 and DMF were successively added at room temperature pure DIEA (2.2 equiv.), a solution in CH_2Cl_2 of acid partner (1.1 equiv.) and a solution in CH_2Cl_2 and DMF of HBTU (1.2 equiv.). After a night to 24 h, the mixture was diluted with aqueous HCl 1M under vigorously stirring (pH = 2). The organic layer was washed with water, aqueous $NaHCO_3$ 0.5M, brine, dried with $MgSO_4$ and the solvents evaporated.

Boc-(Phe-azaPhe-Ala)$_2$-OMe (11a): Purified by the trituration with Et_2O then cyclohexane, yield 92%, white powder, m.p. 132 °C. IR ($CDCl_3$): $\tilde{\nu}_{max}$ = 3438, 3401, 3351, 3259 (NH), 1741, 1700, 1665 (C=O) cm^{-1}. ^1H NMR (300 MHz, CD_3CN): δ = 1.17 (d, J = 7.0 Hz, 3 H, βCH$_3$ Ala), 1.27–1.36 (m, 12 H, βCH$_3$ Ala and Boc), 2.79 (m, 1 H, βCH$_2$ Phe), 2.94–3.00 (m, 2 H, βCH$_2$ Phe), 3.17–3.18 (m, 1 H, βCH$_2$ Phe), 3.60 (s, 3 H, OCH$_3$), 3.91 (m, 1 H, αCH Phe), 4.03–4.05 (m, 1 H, αCH Phe), 4.20–4.25 (m, 2 H, αCH Ala), 4.63 (m, 4 H, CH$_2$, N^{α}-Bn), 5.61 (br. s, 1 H, NHBoc), 6.37 (br. s, 1 H, NH Ala), 6.52 (br. s, 1 H, NH Ala), 7.17–7.28 (m, 21 H, Ar, NH Phe), 8.99 (br. s, 2 H, 2×NH azaPhe) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 17.3 (CH$_3$ Ala), 18.2 (CH$_3$ Ala), 28.6 (3 CH$_3$ Boc), 37.2 (CH$_2$ Phe), 37.4 (CH$_2$ Phe), 50.5 (CH Ala), 52.3 (CH$_2$, N^{α}-Bn), 52.6 (CH Ala, O-CH$_3$), 53.3 (CH$_2$, N^{α}-Bn), 81.3 (C, Boc), 127.8 (CH, Ar), 128.1 (CH, Ar), 128.2 (CH, Ar), 128.4 (CH, Ar), 129.2 (CH, Ar), 129.3 (CH, Ar), 129.5 (CH, Ar), 129.7 (CH, Ar), 129.8 (CH, Ar), 130.3 (CH, Ar), 130.4 (CH, Ar), 137.6 (C, Ar), 138.0 (C, Ar), 138.5 (C, Ar), 139.9 (C, Ar), 158.3 (O=C-NH), 159.7 (O=C-NH), 171.4 (O=C-NH), 175.3 (C=O ester) ppm. HRMS (ESI) calculated for $C_{46}H_{56}N_8$ NaO_9 $[M + Na]^+$ *m/z* 887.4062, found 887.4062.

Boc-(D-Phe-azaPhe-Ala)$_2$-OMe (11b): Purified by the trituration with cyclohexane, yield 95%, white powder, m.p. 172 °C. IR (ATR): $\tilde{\nu}_{max}$ = 3423, 3351, 3240 (NH), 1741, 1704, 1665, 1635 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.30 (d, J = 6.6 Hz, 3 H, βCH$_3$ Ala), 1.34 (s, 9 H, Boc), 1.40 (d, J = 7.0 Hz, 3 H, βCH$_3$ Ala), 2.56–2.60 (m, 1 H, βCH$_2$ Phe), 2.85–2.89 (m, 1 H, βCH$_2$ Phe), 2.98–3.01 (m, 1 H, βCH$_2$ Phe), 3.24–3.27 (m, 1 H, βCH$_2$ Phe), 3.32 (s, 3 H, OCH$_3$), 3.81 (m, 1 H, αCH Ala), 4.10 (m, 1 H, αCH Phe), 4.27–4.34 (m, 2 H, CH$_2$, N^{α}-Bn), 4.40–4.48 (m, 3 H, CH$_2$, N^{α}-Bn, NHBoc and αCH Phe), 4.67–4.68 (m, 1 H, αCH Ala), 5.27 (m, 1 H, CH$_2$, N^{α}-Bn), 6.30 (br. d, J = 8.21 Hz, 1 H, NH Ala), 6.42 (br. s, 1 H, NH Phe), 6.97–7.34 (m, 20 H, Ar), 8.88 (br. s, 1 H, NH azaPhe), 9.48 (br. s, 1 H, NH azaPhe) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.5 (CH$_3$ Ala), 18.9 (CH$_3$ Ala), 29.0 (CH$_3$ Boc), 36.2 (CH$_2$ Phe), 37.1 (CH$_2$ Phe), 49.5 (CH Ala), 52.3 (CH$_2$, N^{α}-Bn), 56.2 (CH Ala, and O-CH$_3$), 82.0 (C, Boc), 127.9 (CH, Ar), 128.1 (CH, Ar), 128.8 (CH, Ar), 129.0 (CH, Ar), 129.5 (CH, Ar), 129.6 (CH, Ar), 136.4 (C Ar), 137.7 (C Ar), 156.9 (O=C-NH), 157.6 (O=C-NH), 170.6 (O=C-NH), 176.4 (C=O ester) ppm. HRMS (ESI) calculated for $C_{46}H_{56}N_8NaO_9$ $[M + Na]^+$ *m/z* 887.4062, found 887.4062.

Supporting Information (see footnote on the first page of this article): ^1H and ^{13}C NMR spectra of **5**, **7**, **8a**, **8b**, **11a**, **11b**, complementary information on structural data, such as chemical shift variations, NOE spectra, NOEs and dihedral angles used for structural calculations and crystals data are available in Supporting Information.

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