CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

Accepted Article

Title: 12/14/14-Helix Formation in 2:1 α/β-hybrid peptides containing bicyclo[2.2.2]octane ring constraints

Authors: Muriel Christiane Amblard; Baptiste Legrand; Christophe André; Laure Moulat; Claude Didierjean; Patrick Hermet; Jean-Louis Bantignies; Jean Martinez; Monique Calmès

To be cited as: Chem. Eur. J. 10.1002/chem.201602746

Link to VoR: http://dx.doi.org/10.1002/chem.201602746

Supported by
ACES

WILEY-VCH

12/14/14-Helix Formation in 2:1 α/β-hybrid peptides containing bicyclo[2.2.2]octane ring constraints

Baptiste Legrand[a], Christophe André[a],Laure Moulat[a], Claude Didierjean[b], Patrick Hermet[c], Jean-Louis Bantignies[d], Jean Martinez[a], Muriel Amblard*[a], Monique Calmès*[a]

[a] Dr. B. Legrand[§], Dr C. André[§],, L. Moulat, Prof. J. Martinez, Dr. M. Calmès, Dr. M. Amblard, Institut des Biomolécules Max Mousseron (IBMM) UMR 5247 CNRS-Université Montpellier-ENSCM- **15 avenue Charles Flahault -** 34093 Montpellier cedex 5, France
E-mail: muriel.amblard@univ-montp1.fr,
monique.calmes@univ-montp2.fr
Homepage: http://www.ibmm.univ-montp1.fr
[b] Dr C. Didierjean, CRM2, UMR 7063 CNRS Université de Lorraine, Boulevard des Aiguilletes, 54506 Vandoeuvre-lès-Nancy Cedex, France
[**c**] Dr P. Hermet**,** Institut Charles Gerhardt Montpellier, UMR-5253, CNRS, Université de Montpellier, ENSCM, Place E. Bataillon, 34095 Montpellier Cédex 5, France
[**d**] Prof. J.L Bantignies**,** Laboratoire Charles Coulomb, UMR 5221 CNRS-Université de Montpellier, 34095 Montpellier, France

§ These authors contributed equally to this work

Supporting information for this article is given via a link at the end of the document

Abstract: The highly constrained β-amino acid ABOC induces different types of helices in β urea and 1:1 α/β amide oligomers. The latter can adopt 11/9- and 18/16-helical folds depending on the chain length in solution. Here we showed that short peptides alternating proteinogenic α-amino acids and ABOC in 2:1 α/β repeat pattern adopted an unprecedented and stable 12/14/14-helix established through extensive NMR, molecular dynamics and IR studies. While the 1:1 α-AA/ABOC helices diverged from the canonical α-helix, the helix formed by the 9-mer 2:1 α/β-peptide allowed the projection of the α-amino acid side chains in a spatial arrangement according to α-helix. Such finding constitutes an important step toward the conception of functional tools, using ABOC residue as a potent helix inducer, for biological applications.

Main text

The propensity of short amino acid sequences with heterogeneous backbones able to adopt helical conformations was first described by the groups of Gellman and Reiser for α/β-hybrid peptides containing a 1:1 alternation of α-amino acids and rigid cyclic β-amino acids, *i.e*. *trans*-ACPC and *cis*-ACC respectively.[1,2] Further studies involving β^3-, $\beta^{2,2}$- or alkyl and cyclic $\beta^{2,3}$-amino acids have significantly extended the chemical and structural diversity of this heterogeneous backbone foldamer family.[3–16] The use of diverse β-amino acids led to a range of helical structures that have been enlarged with α/β-hybrid peptides containing different mode of combinations of α and β residues. However, in this last case a limited set of examples have been reported that can be divided in two groups, i.e. oligomers with repetitive 2:1, 1:2 or 2:2 α:β patterns,[17–20] or oligomers with aperiodic α/β sequence.[21–23] Interestingly, oligomers involving trans-ACPC residue with 2:1 or 1:2 α/β residue patterns adopted distinctive helical structures with the coexistence in each case of two helical forms in solution, whereas in crystal structures only a 10/11/11- and a 11/11/12-helix were observed, respectively.[17,18] More recently, following the proposed stereochemical patterning approach,[19] Martinek and Reiser demonstrated that a 2:2 alternation of cis-ACPC and α-amino acids motifs resulted in a stable 9/12/9/12-helical structure.[20] Quite differently, the group of Balaram observed helical folding in short peptides containing only one or two consecutive acyclic β^3-amino acids in combination with α-amino acid including the strong helix inducing Aib, in both the solid state and in solution.[21,22]

Incorporation of proteogenic or unnatural α-amino acids constitutes a convenient way to generate well-structured hybrid peptides with a large diversity of functional groups. In this context, we recently investigated the structural properties of short oligomers containing both the constrained $\beta^{2,3,3}$-trisubstituted bicyclic amino acid ABOC residue[24] that has showed to promote helical conformations in homo- and mixed oligoureas,[25,26] and proteogenic α-amino acid in a 1:1 alternating pattern.[16] We demonstrated that these α/β-hybrid peptides adopt an 11/9-helix in the solid state, while a conformational polymorphism as a chain-dependent phenomenon was observed in solution. In this study, we propose to extend the conformational analysis within the relatively unexplored 2:1 α/β-hybrid peptides by using the ABOC building block as a potent helix inducer. For that purpose, three 2:1 α/β-hybrid peptides of different lengths (**1**-**3**) containing the periodic trimer Ala-(*S*)-ABOC-Phe were synthesized and their progressive folding evaluated using crystallographic structure analysis, NMR, FT-IR and CD spectroscopies.

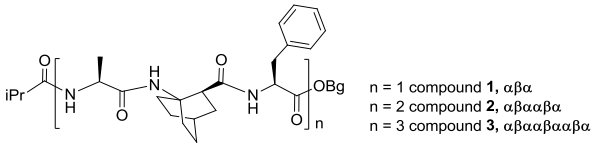

***Figure 1**. Structure of (Ala-(S)-ABOC-Phe)$_n$ α/β-hybrid peptides.*

Each of the α/β-hybrid peptides was prepared in solution by stepwise assembly, using the standard N-Boc protected peptide synthesis strategy (Figure 1, see SI). A N-benzhydrylglycolamide ester group (OBg)[27] was introduced at the C-terminal part of oligomers for crystallization trials and an isobutyryl group was used as capping at the N-terminal part.

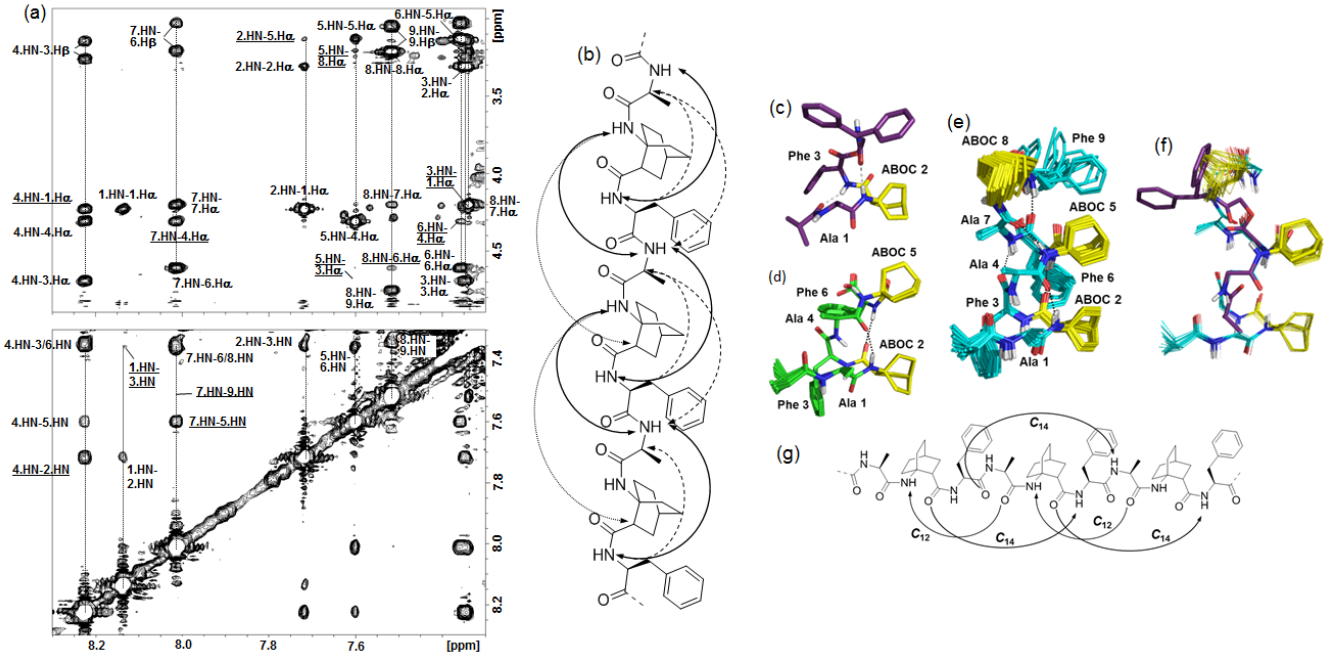

***Figure 2** XRD and NMR studies of the trimer **1**, hexamer **2** and nonamer **3**. (a) HN/Hα and HN/HN-H$_{aro}$ regions of the ROESY spectrum of **3** recorded in CD$_3$OH at 298 K. (b) Typical non-sequential NH$_i$/NH$_{i+2}$ (plain arrows), NH$_i$/Hα$_{i-2}$, NH$_i$/Hα$_{i-3}$ (dashed arrows) and NH$_i$/Hα$_{i+3}$ (dotted arrows) backbone NOE cross-peaks of the (Ala-(S)-ABOC-Phe)$_n$ peptides. (c) Crystal structure of **1**. (d) and (e) superimposition of the 20 lowest energy NMR solution structures of **2** and **3**. (f) Superimposition of the crystal structure of **1** and NMR solution structures of **3**. (g) Characteristic hydrogen-bond pattern stabilizing the (Ala/(S)-ABOC/Phe)$_n$ structures. For the NMR structures, the disordered C-terminal OBg ester and protons were omitted for clarity (hydrogen bonds are represented by dotted lines).*

Attempts to crystallize oligomers **2** and **3** failed, despite the screening of a broad range of solvent conditions and variation of the capping groups such as a C-terminal benzyl ester and a N-terminal Boc or iPrCO groups. However, suitable crystals of the trimer **1** were generated by slow evaporation of a methanol solution (Figure 2c and S1). In the solid state, the trimer **1** exhibited a $C_{11/12}$ turn stabilized by two hydrogen bonds between the CO(iPr) and the NH(Phe), and between the CO(OBg) and the NH(Ala) forming a C_{11} and a C_{12} pseudocycle, respectively. As previously found,[24] ABOC residue displayed a drastically reduced conformational freedom with a θ_2 torsional angle locked around 60° that is favorable for the design of helical foldamers. Other backbone torsional angles for ABOC, Ala and Phe residues are reported in Table 1.

To gain detailed structural information, NMR studies were carried out in CD$_3$OH for the compounds **1**, **2** and **3**. NMR signals were sharps and well-dispersed and all resonances could be assigned using 2D homonuclear COSY, TOCSY, ROESY and heteronuclear ^{15}N- and ^{13}C-HSQC experiments (Figures S3, S4 and Tables S2-S5). It is noteworthy that NOE correlations detected on the ROESY spectra of **1** in CD$_3$OH (Figure S4A) were fully compatible with the crystal structure (Figure 2c). Moreover, although trimer **1** was too short to achieve an helix, its crystal structure superimposed well with the lowest energy NMR solution structures of **3** highlighting the high propensity of short sequences containing ABOC to initiate the folding (Figure 2f). Four characteristic non-sequential backbone NOE correlations NH$_i$/NH$_{i+2}$, NH$_i$/Hα$_{i-2}$, NH$_i$/Hα$_{i-3}$ and NH$_i$/Hα$_{i+3}$ (Figures 2, S4B and S4C), were detected for the 2:1 α/β-hybrid peptides **2** and **3** all along the sequences, providing strong evidences of a conformational preference. Simulated annealing calculations under NOE-derived distance restraints were carried out with AMBER 11 to generate an ensemble of the 20-lowest energy structures for both compounds **2** and **3** (Figure 2). When the flexible C-terminal OBg capping group was omitted, well-defined

structures with low RMSD values were obtained (0.06 and 0.18 for **2** and **3**, respectively) (Table S6). These NMR structure calculations showed the ability of α-AA/ABOC/α-AA oligomers to display a right-handed 12/14/14-helix with 3 residues per turn and a pitch of 5.3 Å. This helix was stabilized by hydrogen bonds with opposite directionality, forming intramolecular 12- and 14-membered pseudocycles. The 12-membered ring involved the CO_i(Ala) and the NH_{i-2}(ABOC) with backward hydrogen-bond orientation, while the two 14-membered rings involved the CO_i(ABOC and Phe) and the NH_{i+4}(Phe and Ala) with forward hydrogen-bond orientation.

The φ, θ and ψ average values in compounds **2** and **3** for ABOC were 61°, 50°, and -100° respectively, whereas φ = -130° and ψ = 157° for the Ala residue, and φ = -61° and ϕ = -41° for the Phe residue (Table 1). Such dihedral angle values were similar to those measured on the trimer **1** solid-state structure, except for the φ angle value of the first residue (Tables 1 and S7).

Table 1. Backbone torsion angle values

α/β-hybrid peptides	Residue	ϕ	θ	ψ
Predicted H12/14/14* (average values)	α-AA	-90 ± 21		113 ± 15
	β-AA	75 ± 16	64 ± 14	-119 ± 23
	α-AA	-83 ± 31		-24 ± 35
2 and **3** NMR structures (average values)	α-AA	-130 ± 24		157 ± 10
	β-AA	61 ± 9	50 ± 7	-100 ± 5
	α-AA	-61 ± 14		-41 ± 18
Trimer **1** XRD structure	α-AA	-59		148
	β-AA	75	63	-82
	α-AA	-87		-66

[a] Calculated at the HF/6-31G* level of Ab Initio MO Theory for β/α/α-hydrid octamers.[28]

We then recorded mid infrared absorption spectra (4000-400 cm^{-1}) of the compounds **1**, **2** and **3** in $CHCl_3$ to assess their ability to establish intramolecular hydrogen bond networks monitoring the vibrational frequencies of both amide A (3600-3100 cm^{-1}) and amide I (1800-1600 cm^{-1}). As expected, the three oligomers showed similar infrared active bands (Figures 3b and 3c). They exhibited three main contributions in the amide A region at high (3504 cm^{-1} and 3399 cm^{-1}) and low frequencies (from 3348 to 3306 cm^{-1}) corresponding to free and hydrogen bonded amide, respectively. In the amide I region, while the high frequency band around 1742 cm^{-1} was assigned to the ester C=O group,[29] the large band centred around 1660 cm^{-1} corresponded to the free and H-bonded amide I features. In both amide A and amide I regions, the main band intensities increased with the oligomer size according to the typical hydrogen bonds pattern stabilizing the 12/14/14-helix solved by NMR.

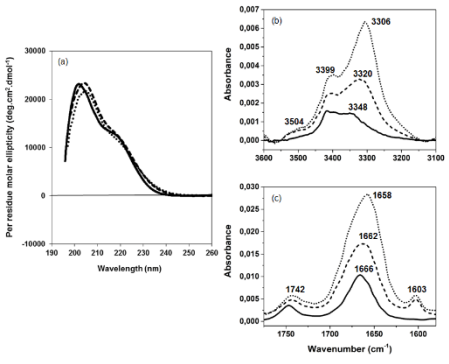

***Figure 3**. (a) CD spectra in CH_3OH (100 µM) at 20°C and (b-c) FT-IR spectra (2 mM) in $CHCl_3$ at 20°C (b) Amide A and (c) Amide I region. Compounds **1**, **2** and **3** are indicated by plain, dashed and dotted lines, respectively.*

Interestingly, we could notice a significant downshift of the H-bonded amide A and amide I vibrations to the lower frequencies (from 3348 to 3306 cm^{-1} and 1666 to 1658 cm^{-1}) with the oligomers length. These data supported the progressive strengthening of the hydrogen bonds network with the extension of the oligomers (Figures 3b and 3c). We also performed DFT calculations starting from the optimized lowest energy NMR structure of compound **3** to calculate the FT-IR spectrum of the 12/14/14 helical fold of the hexamer (see SI). A good correlation between the experimental and the calculated vibrational features was obtained, validating the

helical conformational preference of **3** and allowing us to identify the amide modes from the different residues (Figure S6 and Table S8).

At last, according to their similar folds, the three oligomers **1**, **2** and **3** shared comparable CD profiles with a strong maximum at around 205 nm and a shoulder at 220 nm (Figures 3a and S7). The positive Cotton effects observed were consistent with a right-handed helical fold.

Overall, these results showed the ability of 2:1 α/β-hybrid peptides to adopt a well-defined secondary structure. Interestingly, the 12/14/14-helix characterized in this study has been previously predicted to be favored by theoretical calculations[28] among the β/α/α-hydrid octamers, but never obtained before (Table 1). NMR structures of **3** superimposed well with the predicted structure of β/α/α-hydrid peptides (Figure S5). In addition, it could be noticed that the ABOC backbone torsional angles were nearly similar to those previously observed within the 12/14 helix of the ABOC-based oligoureas[25,26] and also within the 11/9- and 18/16-helices of the 1:1 α-AA/ABOC oligoamides[16] emphasizing its high conformational restriction. The 1:1 and 2:1 α-AA/ABOC-peptide helices exhibited different helical parameters which affect their stability and the relative distribution of the ABOC and α residues along the helices axis (Figure 4). Unlike the 1:1 α-AA/ABOC alternating pattern[16] but also the 2:1 α/β-peptides reported by the group of Gellman,[17] no helical polymorphism was observed in solution among the 2:1 α-AA/ABOC-hybrid peptides. While the 11/9-helix the 18/16-helix have a pitch of 6.2 and 5.0 and a diameter of 4.0 and 7.5 Å, respectively, the 12/14/14-helix possess properties closer than those of the α-helix, with a rise per turn of ca 5.3 Å and a diameter of 5.5 Å. Thus, as shown in Figure 4, a good overlap was observed between the central α-residues of peptides **3** and the corresponding residues of the model canonical α-helix (rmsd values on the Cα atoms of the α-residues was 1.35 and dropped to 0.35 Å when the N- and C-terminal fraying residue were omitted). Both helices shared comparable projection of their side chains with only small deviations at positions i+4 and i+7 (rmsd value on the Cα-Cβ atoms was 0.55 Å when the N and C terminal residues were omitted).

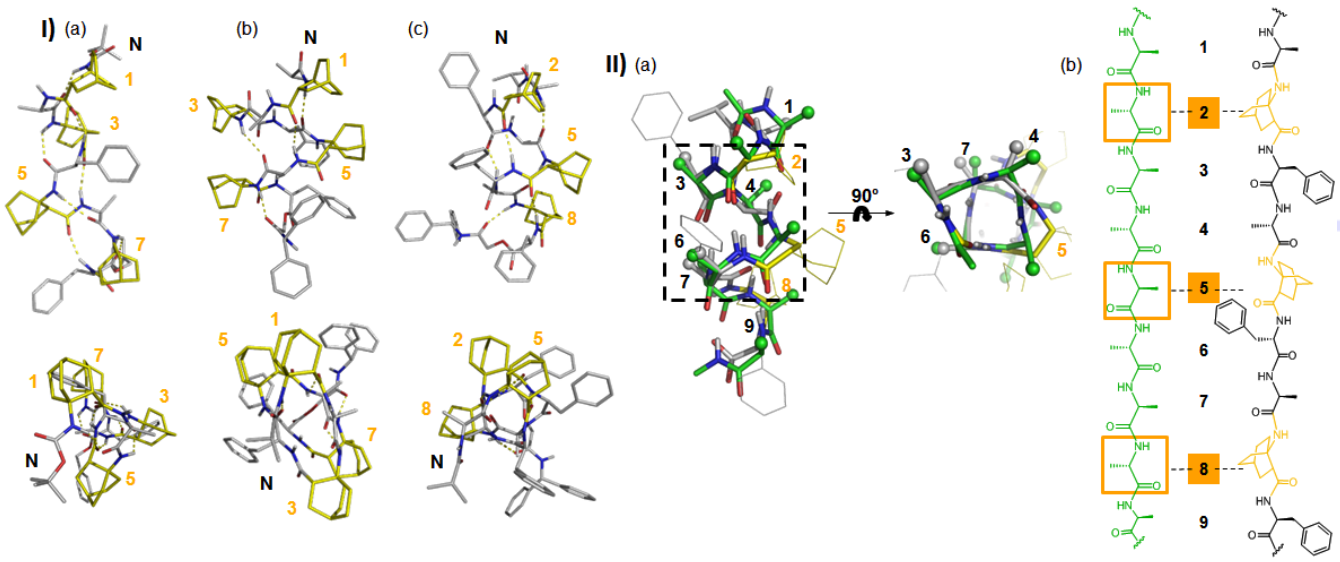

***Figure 4**. **I)** Comparison of 1:1 and 2:1 α-AA/ABOC-hybrid peptides structures: (a) X-ray crystal structure of the 11/9-helix in 1:1 octamer; (b) and (c) NMR solution structures of the 18/16-helix in 1:1 octamer and the 12/14/14-helix in 2:1 nonamer, respectively. Except amides, protons were omitted for clarity (Hydrogen bonds are represented by dotted lines); **II)** Superimposition of the 12/14/14 Ala/(S)-ABOC/Phe helix (in grey/yellow) and the poly-Ala α-helix (in green): (a) transversal and axial views, b) linear sequences of the poly-Ala and 9-mer α/β/α peptide.*

In conclusion, we established the conformational preference of 2:1 α/β-hybrid peptides incorporating the ABOC motif as constrained β-residue and confirmed its strong ability to drive helical folding in peptide sequences. We demonstrated that the 2:1 α-AA/ABOC displayed an original and predictable 12/14/14 helical fold that would match with the α-helix unlike the 1:1 α-AA/ABOC helical folds. In particular, the 2:1 alternating pattern affords a helical scaffold projecting α-AA side chains in comparable positions as certain side chains of the canonical α-helix. Thus, considering the distribution of the ABOC and the α-amino acids along the 12/14/14-helix axis, we are currently focusing our attention on these new helical scaffolds for biological applications.

Acknowledgements. We thank the CNRS, ANR-10-LABX-05-01 and the Université de Montpellier for financial support. We also thank SIMS platform, LMP (Laboratoire de Mesures Physiques) for providing access to IR, NMR and CD facilities and Université de Lorraine for XRD facilities.

References

[1] A. Hayen, M. A. Schmitt, F. N. Ngassa, K. A. Thomasson, S. H. Gellman, *Angew. Chem.-Int. Ed.* **2004**, *43*, 505–510.
[2] S. De Pol, C. Zorn, C. D. Klein, O. Zerbe, O. Reiser, *Angew. Chem.-Int. Ed.* **2004**, *43*, 511–514.
[3] W. S. Horne, S. H. Gellman, *Acc. Chem. Res.* **2008**, *41*, 1399–1408.
[4] P. G. Vasudev, S. Chatterjee, N. Shamala, P. Balaram, *Chem. Rev.* **2011**, *111*, 657–687.
[5] T. A. Martinek, F. Fulop, *Chem. Soc. Rev.* **2012**, *41*, 687–702.
[6] G. V. M. Sharma, P. Nagendar, P. Jayaprakash, P. R. Krishna, K. V. S. Ramakrishna, A. C. Kunwar, *Angew. Chem.-Int. Ed.* **2005**, *44*, 5878–5882.
[7] G. V. M. Sharma, T. A. Yadav, M. Choudhary, A. C. Kunwar, *J. Org. Chem.* **2012**, *77*, 6834–6848.
[8] D. Seebach, B. Jaun, R. Sebesta, R. I. Mathad, O. Flögel, M. Limbach, H. Sellner, S. Cottens, *Helv. Chim. Acta* **2006**, *89*, 1801–1825.
[9] K. Basuroy, V. Karuppiah, N. Shamala, P. Balaram, *Helv. Chim. Acta* **2012**, *95*, 2589–2603.
[10] K. Basuroy, V. Karuppiah, P. Balaram, *Org. Lett.* **2014**, *16*, 4614–4617.
[11] D. Balamurugan, K. M. Muraleedharan, *Chem. – Eur. J.* **2012**, *18*, 9516–9520.
[12] S. H. Choi, I. A. Guzei, L. C. Spencer, S. H. Gellman, *J. Am. Chem. Soc.* **2008**, *130*, 6544–6550.
[13] G. V. M. Sharma, T. Sridhar, B. Veena, P. P. Reddy, S. V. Reddy, C. Bruneau, A. C. Kunwar, *New J. Chem.* **2015**, *39*, 3295–3309.
[14] G. V. M. Sharma, T. Sridhar, P. P. Reddy, A. C. Kunwar, *Eur. J. Org. Chem.* **2013**, 3543–3554.
[15] M. Lee, J. Shim, P. Kang, I. A. Guzei, S. H. Choi, *Angew. Chem.-Int. Ed.* **2013**, *52*, 12564–12567.
[16] B. Legrand, C. André, L. Moulat, E. Wenger, C. Didierjean, E. Aubert, M. C. Averlant-Petit, J. Martinez, M. Calmes, M. Amblard, *Angew. Chem. Int. Ed.* **2014**, *53*, 13131–13135.
[17] M. A. Schmitt, S. H. Choi, I. A. Guzei, S. H. Gellman, *J. Am. Chem. Soc.* **2006**, *128*, 4538–4539.
[18] S. H. Choi, I. A. Guzei, L. C. Spencer, S. H. Gellman, *J. Am. Chem. Soc.* **2009**, *131*, 2917–2924.
[19] I. M. Mandity, E. Weber, T. A. Martinek, G. Olajos, G. K. Toth, E. Vass, F. Fulop, *Angew. Chem.-Int. Ed.* **2009**, *48*, 2171–2175.
[20] L. Berlicki, L. Pilsl, E. Weber, I. M. Mandity, C. Cabrele, T. A. Martinek, F. Fulop, O. Reiser, *Angew. Chem.-Int. Ed.* **2012**, *51*, 2208–2212.
[21] R. S. Roy, I. L. Karle, S. Raghothama, P. Balaram, *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 16478–16482.
[22] K. Ananda, P. G. Vasudev, A. Sengupta, K. M. P. Raja, N. Shamala, P. Balaram, *J. Am. Chem. Soc.* **2005**, *127*, 16668–16674.
[23] G. V. Sharma, N. Chandramouli, S. J. Basha, P. Nagendar, K. V. Ramakrishna, A. V. Sarma, *Chem Asian J* **2011**, *6*, 84–97.
[24] C. André, B. Legrand, C. Deng, C. Didierjean, G. Pickaert, J. Martinez, M. C. Averlant-Petit, M. Amblard, M. Calmes, *Org. Lett.* **2012**, *14*, 960–963.
[25] B. Legrand, C. André, E. Wenger, C. Didierjean, M. C. Averlant-Petit, J. Martinez, M. Calmes, M. Amblard, *Angew. Chem. Int. Ed.* **2012**, *51*, 11267–11270.
[26] C. André, B. Legrand, L. Moulat, E. Wenger, C. Didierjean, E. Aubert, M. C. Averlant-Petit, J. Martinez, M. Amblard, M. Calmes, *Chem. – Eur. J.* **2013**, *19*, 16963–16971.
[27] M. Amblard, M. Rodriguez, J. Martinez, *Tetrahedron* **1988**, *44*, 5101–5108.
[28] P. Schramm, G. V. M. Sharma, H. J. Hofmann, *Biopolymers* **2010**, *94*, 279–291.
[29] V. Martin, B. Legrand, L. L. Vezenkov, M. Berthet, G. Subra, M. Calmès, J.-L. Bantignies, J. Martinez, M. Amblard, *Angew. Chem. Int. Ed Engl.* **2015**, *54*, 13966–13970.

Keywords: bicyclic β-amino acid • 12/14/14-helix • α/β-hybrid peptides • structural determination • helical structure

Table of Contents

α/β-hybrid peptides combining 2:1 α/β pattern of the highly constrained ABOC residue and proteogenic α-amino acids adopted an unprecedent 12/14/14-helix in solution. No helical polymorphism was observed for this helix. The 9-mer α/β/α peptide showed some similarities in α-AA side chain projection with that of α-helix.