Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 1787–1790

Molecular modeling study for a novel structured oligomer subunit selection: the example of 2-aminomethyl-phenyl-acetic acid

Nicolas Raynal,[a] Marie-Christine Averlant-Petit,[b] Gilbert Bergé,[a] Claude Didierjean,[c] Michel Marraud,[b] Christiane Duru,[a] Jean Martinez[a] and Muriel Amblard[a,*]

[a]*UMR-CNRS 5810, Université de Montpellier I et II, Montpellier Cedex 05, France*
[b]*UMR CNRS-INPL 7568, ENSIC, BP 451, 54001 Nancy Cedex, France*
[c]*UMR CNRS-UHP 7036, Faculté des Sciences, BP 259, 54506 Vandoeuvre Cedex, France*

Received 31 October 2006; revised 21 December 2006; accepted 5 January 2007
Available online 10 January 2007

Abstract—Oligomers derived from dipeptide mimics were selected by computational study for their suitability to fold in ordered structures. After selection of a monomeric unit, short oligomers were synthesized and analyzed by NMR and IR. Oligomers built from 2-aminomethyl-phenyl-acetic acid were shown to adopt a helical structure stabilized by 10-membered ring hydrogen bonds.

α-Helices are widely involved in protein–protein, protein–nucleic acid recognition, and binding processes. Identification of unnatural helical oligomers, stable toward enzymatic degradation, that present the structural and biological characteristics of biopolymers has been the focus of extensive research over the last 10 years in several laboratories. They constitute attractive structures for understanding molecular assembly and protein folding as well as for designing new classes of drugs and new biomaterials. The first studies by Gellman and Seebach groups have shown that oligomers derived from β-amino acids (β-peptides), called 'foldamers', can adopt a well-defined secondary structure.[1–3] Today, several other oligomers have been described to fold into a specific helical secondary structure.[4] Our efforts were aimed at identifying new oligomers derived from dipeptide mimics for their suitability as 'foldamer' building blocks. Our research was motivated by a molecular modeling analysis of oligomers constructed from various dipeptide mimics described in the literature.[5,6] This study showed that their most stable conformers possessed a strong helix-forming propensity.[7] In order to validate our molecular modeling protocol, it was applied to the oligomers of (2*R*,3*S*,4*R*)-3-amino-4-[(1*S*)-1-hydroxyethyl]oxetane-2-carboxylic acid and (*R*,*R*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC) described in the literature.[8,9] In both cases, the 10-helical and 12-helical conformations obtained by molecular modeling were in agreement with the experimental results obtained by the groups of Fleet and Gellman.

The first step of our study was to establish a selection of rigidified motifs. For this purpose, we virtually constructed decamers of these motifs that we analyzed by molecular modeling for structural prediction.[10] After selection of motifs likely to adopt helix-like structures, we synthesized the corresponding oligomers of various lengths for ^{1}H NMR and IR studies.

Herein, we report NMR and IR studies of short oligomers of one of the first motives that we have selected as potential novel structured oligomer subunits, the 2-aminomethyl-phenyl-acetic acid motif (AMPA). Molecular modeling analysis showed that the predicted lowest energy structure of the Ac-$(AMPA)_{10}$-NHMe oligomer adopts a helical structure defined by a 10-membered ring hydrogen bond between a carbonyl at position *i* and an amide proton at position $i+2$ (Fig. 1).

Due to the lack of chiral center, the oligomer has no helical handedness (the right-handed helix was presented). This dipeptide mimetic helix has approximately 3.36 residues per turn and a pitch of about 10.4 Å. The most stable helical structure is represented in Figure 1a.

Keywords: Helical oligomers; ^{1}H NMR and IR studies; Molecular modeling; Dipeptide mimics; Poly(AMPA).
* Corresponding author. Tel./fax: +33 4 67 54 86 37; e-mail: muriel.amblard@univ-montp1.fr

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.01.032

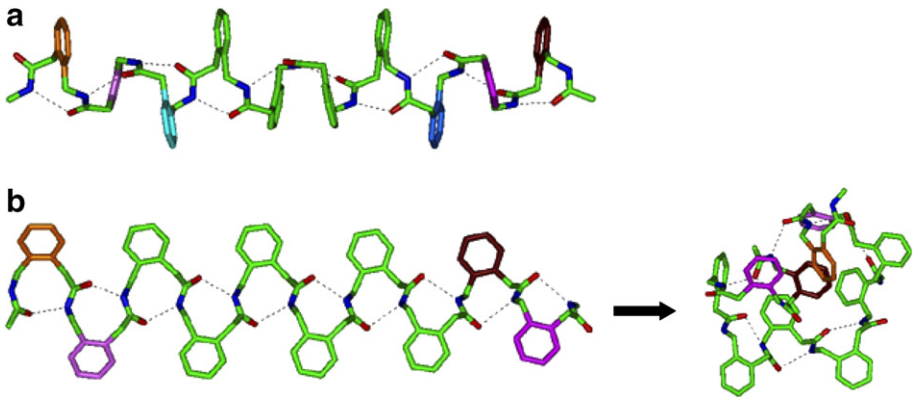


Figure 1. Computational conformation of Ac-$(AMPA)_{10}$-NH-Me oligomers with trans (a) or syn (b) orientation of the amide group.

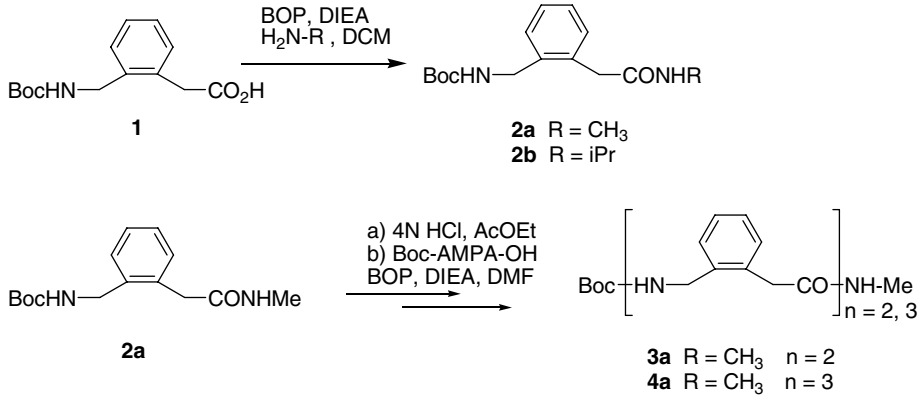
The molecular modeling prediction that oligomers of AMPA could adopt a 10-helix secondary structure remained to be experimentally verified. We undertook NMR and IR studies of different AMPA oligomers. We prepared derivatized AMPA dimers and trimers, Boc-$(AMPA)_2$-NH-Me (**3a**), Boc-$(AMPA)_3$-NH-Me (**4a**). NMR and IR analyses of the Ac-$(AMPA)_{10}$-NH-Me oligomer, that we used for molecular modeling prediction, were not possible for solubility problems.

The precursor monomer *N*-Boc-protected 2-aminomethyl-phenyl-acetic acid (Boc-AMPA-OH, **1**) was prepared according to the method described by Zhilian and Pelletier.[11] Condensation of Boc-AMPA-OH with methylamine or isopropylamine was carried out through activation with BOP in the presence of DIEA to afford the corresponding Boc-AMPA-NHMe (**2a**) and Boc-AMPA-NH*i*Pr (**2b**) in quantitative yields (Scheme 1). Boc removal by a 4 N HCl solution in AcOEt followed by successive coupling to Boc-AMPA-OH with BOP reagent in the presence of DIEA afforded compounds **3a** and **4a**.

To determine the conformational properties induced by the increasing number of AMPA motives, we have examined the IR (NH and CO stretching) and proton NMR data (NH solvent accessibility) for Boc-$(AMPA)_n$-NHR (R = Me or *i*Pr) oligomers, $n = 1$–3. Higher oligomers could not be investigated due to their low solubility in poor solvating medium (CH_2Cl_2, $CHCl_3$) required for intramolecular H-bonding.

The IR spectra of monomers **2a** and **2b**, and especially the balance between the high frequency domain (3400–3500 cm^{-1}) typical of free NHs and the low frequency domain (3250–3400 cm^{-1}) typical of bonded NHs, strongly depend on the solvent (CH_2Cl_2 or CCl_4). Although CH_2Cl_2 is considered as a very poor solvating medium favoring intramolecular H-bonds, the small intensity of the wide absorption at 3356 cm^{-1} (**2a**) or 3339 cm^{-1} (**2b**) indicated a small percentage of NH to CO H-bonding. The sensitivity of this absorption is relative to the nature of the C-terminus, that is, **2a** (R = NHMe) or **2b** (R = NH*i*Pr). This indicates that the amide proton involved in the hydrogen bond is the C-terminal NH (vs the Boc-NH).[12,13] In CCl_4, the low frequency absorptions at 3372 cm^{-1} (**2a**) or 3354 cm^{-1} (**2b**) were much more intense and indicated a higher percentage of folded molecules. Moreover, the Boc-CO absorption clearly gave two components at 1716 and 1703 cm^{-1} (**2a**) with nearly equal intensity, confirming the occurrence of the NHR to Boc-CO H-bond for about half of the molecules.

X-ray crystallographic structure of the monomeric unit Boc-AMPA-NH*i*Pr **2b**[14] revealed an extended molecule exchanging two intermolecular urethane NH to urethane CO (NH···O = 2.93 Å), and amide NH to amide



Scheme 1. Boc$(AMPA)_n$-NHMe synthesis.

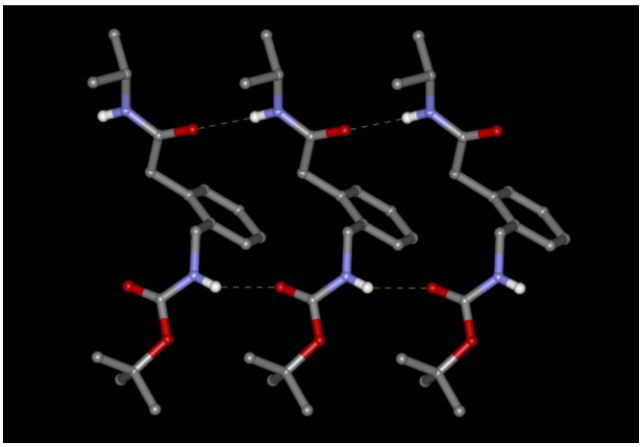


Figure 2. View of the crystal packing of the extended molecular structure of **2b**. NH···O Hydrogen bonds, indicated by dashed lines, join molecules into infinite chains in the [1 0 0] direction. The isopropyl group is disordered over three sites with occupancy factors 0.48, 0.32, and 0.20. Only one component is shown. The aliphatic and aromatic hydrogens have been omitted for clarity.

CO (NH···O = 3.08 Å) H-bonds with its neighbors. The amide groups are nearly trans-planar with standard dimensions, and are in an anti orientation with reference to the planar aromatic group (Fig. 2).

The AMPA motif is therefore intrinsically flexible and capable of adopting an extended conformation in the solid state or in a solvating medium, and a folded conformation in an inert medium. A molecular modeling analysis of **2a** showed that the H-bond stabilizing the putative folded conformation closes a 10-membered pseudocycle with trans or syn orientation of the amide group (Fig. 3). Both in the predicted lowest energy conformer of Ac-$(AMPA)_{10}$-NHMe and in the Boc-AMPA-NH*i*Pr crystal, even though no intramolecular H-bonds were observed, the monomeric unit amide group, with reference to the planar phenyl ring, adopted a trans orientation. However, we have no experimental evidence for the actual trans or syn orientation of the folded conformation in solution. The syn orientation is similar to the so-called βVI-turn found in peptide sequences containing an intra-chain N-substituted amide link.[15,16] This prompted us to examine the propensity of oligomer to have the amide group into a syn orientation (Fig. 1b).

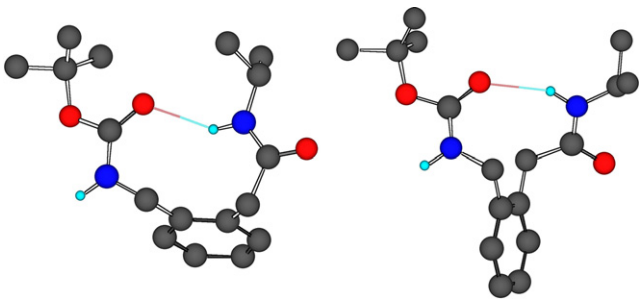


Figure 3. Two putative folded conformations of **2a** with an intramolecular H-bond closing a 10-membered pseudocycle, in a syn (a) or an anti (b) orientation of the amide groups with reference to the phenyl ring (energy minimization using MOPAC program in CS Chem3D). The aliphatic and aromatic hydrogens have been omitted for clarity.

The number n of AMPA motifs in Boc-$(AMPA)_n$-NHR oligomers induces noticeable changes in the low frequency domain of the NH stretching absorption (Fig. 4). Its intensity increases with n, and the maximum of the absorption is progressively shifted to lower frequencies. In fact, two maxima are observed at about 3350 and 3280 cm^{-1}. The former, which is observed for **2a**, is progressively embedded in the latter for **3a** and even more for **4a**.

The solvent sensitivity of the NH proton resonances has been measured in $CDCl_3$/DMSO-d_6 mixtures with increasing DMSO-d_6 content for oligomers **3a** and **4a** (Fig. 5). Both N- and C-terminal NHs are solvent accessible and mostly free of H-bonding, even in $CDCl_3$. On the contrary, the NHs between two AMPA-motifs[17] are solvent protected and engaged in intramolecular H-bonding. The fact that one NH in **3a** and two NHs in **4a** share the same behavior strongly suggests that this H-bond closes a 10-membered pseudocycle, exactly as

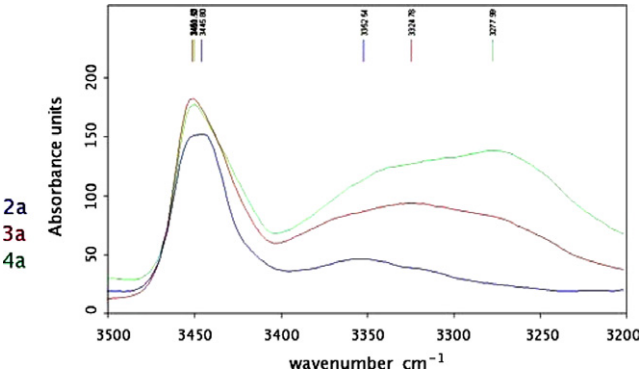


Figure 4. Influence of the number n of AMPA motifs on the NH stretching absorption for oligomers **2a**, **3a**, and **4a** ($n = 1$, 2, and 3, respectively) in CH_2Cl_2.

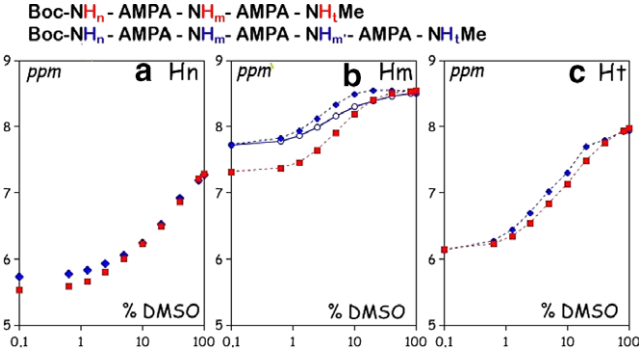


Figure 5. Influence of DMSO-d_6 volumetric percentage in $CDCl_3$/DMSO-d_6 mixtures (logarithmic scale) on the NH proton resonances for the Boc-$(AMPA)_n$-NHMe oligomers with $n = 2$ (red) and 3 (blue). The left graph refers to the N-terminal Boc-NH group, the right one to the C-terminal NHMe group, and the middle one to the NH group between two X motifs.

for the minor folded conformation of **2a**. The low frequency observed for **4a**, in comparison with **2a**, may be the consequence of the double involvement, both of its NH and CO groups in an H-bonding.[18] Luppi et al. raised such results with the oligomers of oxazolidinone derivatives.[19] These data suggested that there is an increase of hydrogen bonds with the oligomer size suggesting that short oligomers of AMPA are able to fold in an ordered structure. The propensity of short oligomers to fold in organized structure was observed in foldamers.[1–4,19]

However, as discussed before, in the absence of further experimental data on the syn and anti orientations present in the AMPA oligomer, we constructed the two possible helices (Fig. 1) and we compared their stability by computational analysis. Among the conformations of lowest energy, those presenting a syn orientation of the amide group contained hydrogen bond between the CO and NH of the same AMPA residue resulting in an eight-membered ring. However, this computational prediction did not fit with the experimental IR data showing a C═O–H–N hydrogen in the N- to C-terminal direction forming a 10-membered ring. The helix with a 10-membered ring hydrogen bond and a syn amide group orientation observed in the transition state (Fig. 1b), led after molecular modeling minimization to an unstructured oligomer. These different results prompted us to consider that the preferred structure adopted a 10-helix with the amide groups in a trans orientation.

In summary, we have shown by NMR and IR studies that the short oligomers of 2-aminomethyl-phenyl-acetic acid, selected by computational analysis for their possibility to induce a well-defined secondary structure, could adopt a 10-helical folding pattern. In the absence of chiral center within the molecule and the difficulty of analyzing homo-oligomers, the availability of syn and anti orientations of the amide was analyzed by molecular modeling. The anti orientation of the amide group that was also observed in the crystal of the AMPA derivative seems to be the preferred conformation.

This study suggested that computational analysis can allow us to establish a pre-selection of dipeptide mimetic to construct new families of foldamers. The use of AMPA oligomers as templates to design more functionalized and soluble oligomers is under investigation in our laboratory.

Supplementary data

Experimental procedures, NMR, HPLC, and mass spectrometry data, and **2b** X-ray data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.01.032.

References and notes

1. Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180.
2. Cheng, R. P.; Gellman, S. H.; Degrado, W. F. *Chem. Rev.* **2001**, *101*, 3219–3232.
3. Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015–2022.
4. Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4011.
5. Gillespie, P.; Cicariello, J.; Olson, G. L. *Peptide Sci.* **1997**, *43*, 191–217.
6. Obrecht, D.; Altorfer, M.; Robinson, J. A. *Adv. Med. Chem.* **1999**, 1–68.
7. Amblard, M.; Martinez, J.; Bergé, G. Patent 11/01/2000 sous le n° 0000288, WO B0384.
8. Claridge, T. D. W.; Goodman, J. M.; Moreno, A.; Angus, D.; Barker, S. F.; Taillefumier, C.; Watterson, M. P.; Fleet, G. W. J. *Tetrahedron Lett.* **2001**, *42*, 4251–4255.
9. Applequist, J.; Bode, K. A.; Appella, D. H.; Christianson, L. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4891–4892.
10. Experiments were performed on silicon graphics using Discover Molecular Simulation program, Biosym/MSI. We first performed a molecular dynamic at 1000 K and a minimization of the monomer under its aldehydic form H-AMPA-H. The conformer of the lowest energy was selected and its corresponding Ac-AMPA-NH-Me was subjected to a 45° variation of the four dihedral angles. After minimization, we selected 10 conformations corresponding to the predicted lower energy conformation (34.33 kcal/mol) and those having an energy of 34.33 kcal plus an increment equal to or less than 5 kcal/mol.
11. Zhilian, T.; Pelletier, J. C. *Tetrahedron Lett.* **1998**, *39*, 4773–4776.
12. Aubry, A.; Cung, M. T.; Marraud, M. *J. Am. Chem. Soc.* **1985**, *107*, 7640–7647.
13. Jiménez, A. I.; Cativiela, C.; Aubry, A.; Marraud, M. *J. Am. Chem. Soc.* **1998**, *120*, 9452–9459.
14. *Crystal data* **1b**: $C_{17}H_{26}N_2O_3$, $M_r = 306.40$, colorless prism, crystal size $0.2 \times 0.1 \times 0.1\ mm^3$, $a = 5.1411(4)$ Å, $b = 11.5197(10)$ Å, $c = 15.236(2)$ Å, $\alpha = 82.930(3)°$, $\beta = 89.537(2)°$, $\gamma = 78.520(7)°$, $V = 877.42(15)$ Å^3, $T = 293$ K, triclinic, space group $P\bar{1}$, $Z = 2$, $D_c = 1.160\ g\ cm^{-3}$, $\mu = 0.079\ mm^{-1}$, $\lambda = 0.71073$ Å. 4323 reflections collected, 2474 independent (Rint = 0.0340) and 1394 observed reflections, 203 refined parameters, $R = 0.064$, $wR2 = 0.125$. CCDC-633961 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
15. Aubry, A.; Vitoux, B.; Marraud, M. *J. Chim. Phys., Phys. Chim. Biol.* **1985**, *82*, 933–939.
16. Muller, G.; Gurrath, M.; Kurz, M.; Kessler, H. *Proteins* **1993**, *15*, 235–251.
17. Due to the superposed proton resonances for the Ph–CH_2 protons in **3a**, and to the absence of Overhauser effects across X motifs, the middle NHs in **3a** could not be discriminated.
18. Cung, M. T.; Marraud, M.; Neel, J.; Aubry, A. *Biopolymers* **1978**, *17*, 1149–1173.
19. Luppi, G.; Soffrè, C.; Tomasini, C. *Tetrahedron: Asymmetry* **2004**, *15*, 1645–1650.