Formaggio Fernando (Orcid ID: 0000-0003-2008-4961)

De Zotti Marta (Orcid ID: 0000-0002-3302-6499)

Total synthesis of the natural, medium-length, peptaibol pentadecaibin and study of the chemical features responsible for its membrane activity

Laura Morbiato[1], David S. Haneen[2], Fernando Formaggio[1,3], Marta De Zotti[1,3,*]

[1]Department of Chemical Sciences, University of Padova, Padova, Italy

[2]Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

[3]Padova Unit, CNR Institute of Biomolecular Chemistry, University of Padova, Padova, Italy

Correspondence:

Marta De Zotti, Department of Chemical Sciences, University of Padova, 35131 Padova, Italy.

Email: marta.dezotti@unipd.it

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/psc.3479

Abstract

Peptaibols are naturally-occurring, antimicrobial peptides endowed with well-defined helical conformations and resistance to proteolysis. Both features stem from the presence in their sequence of several, C^{α}-tetrasubstituted, α-aminoisobutyric acid (Aib) residues. Peptaibols interact with biological membranes, usually causing their leakage. All of the peptaibol-membrane interaction mechanisms proposed so far begin with peptide aggregation or accumulation. The long-length alamethicin, the most studied peptaibol, acts by forming pores in the membranes. Conversely, the carpet mechanism has been claimed for short-length peptaibols, such as trichogin. The mechanism of medium-length peptaibols is far less studied and this is partly due to the difficulties of their synthesis. They are believed to perturb membrane permeability in different ways, depending on the membrane properties. The present work focuses on pentadecaibin, a recently discovered, medium-length peptaibol. In contrast to the majority of its family members, its sequence does not comprise hydroxyprolines or prolines, and its helix is not kinked. A reliable and effective synthesis procedure is described that allowed us to produce also two shorter analogs. By a combination of techniques, we were able to establish a 3D-structure-activity relationship. In particular, the membrane activity of pentadecaibin heavily depends on the presence of three consecutive Aib residues that are responsible for the clear, albeit modest, amphiphilic character of its helix. The shortest analog, devoid of two of these three Aib residues, preserves a well-defined helical conformation, but not its amphipathicity, and loses almost completely the ability to cause membrane leakage. We conclude that pentadecaibin amphiphilicity is probably needed for the peptide ability to perturb model membranes.

KEYWORDS: amphiphilicity; helical conformation; peptaibol; peptide-membrane interaction

1 INTRODUCTION

Peptaibols are a family of natural antimicrobial peptides featuring several α-aminoisobutyric acid (Aib) residues and a C-terminal 1,2-aminoalcohol in their sequences, hence their name (Pept*aib*ol) [1]. Peptaibols are proteolysis resistant [1,2] and membrane active [3]. They interact effectively with phospholipid membranes, causing their leakage through mechanisms not yet fully understood, although two general mechanisms have been observed [4]: the carpet mechanism, typically adopted by short-length (about 10 residues) peptaibols, such as trichogin GA IV [5], and (ii) the barrel-stave mechanism, observed with long-length (about 20 residues) peptaibols, such as alamethicin [6]. The medium-length peptaibols [7], typically made up of 13-15 amino acid residues, are the least studied. They are supposed to switch between the two aforementioned mechanisms of peptide-membrane interaction in response to membrane composition and thickness [8]. Their wavering between mechanisms suggests a kind of selective bioactivity, which is promising for their application as antimicrobial or antitumor agents. Indeed, some medium-length peptaibols were reported to display antitumor activity [9]. The medium-length heptaibin showed also selectivity towards negative-charged liposomes, a simple model for cancer cell membranes, over zwitterionic ones that mimic healthy cells/erythrocytes [7]. Further studies on such a promising class of natural bioactive peptides are hampered by a lack of effective synthetic procedures, since the presence of several Aib residues in a row often results in unsuccessful couplings and the production of deleted sequences. In this work, we focus on the recently discovered medium-length peptaibol pentadecaibin [10]. We investigate how the stretch of three consecutive Aib, a common feature of medium-length peptaibols, affects the peptide 3D-structure and membrane interaction. We also propose a straightforward procedure for its synthesis. The reported study with model liposomes points out that the –$(Aib)_3$- stretch is needed for a successful, pentadecaibin-mediated, membrane leakage.

2 MATERIAL AND METHODS

2.1 Materials

Phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, and cholesterol were purchased from Avanti Polar Lipids (AL, USA). Fmoc-protected amino acids, TFFH, and solvents for peptide synthesis were purchased from Sigma-Aldrich. Oxyma-pure and diisopropylcarbodiimide (DIC) were acquired from IRIS Biotech. Unless otherwise stated, all other reagents were purchased from Merck (MA, USA).

2.2 Peptide synthesis and purification

The peptides were synthesized by manual solid phase synthesis, starting from a 2-chlorotrityl resin, preloaded with L-phenylalaninol (loading: 0.4 mmol/g) and using *N*,*N*-dimethylformamide (DMF) as solvent. The single coupling steps, not involving Aib residues (Gly^2, Ala^3, and Gln^{14}), were performed with 3 equivalents of the incoming, activated amino acid residue. Aib^1 was acetylated twice [acetic anhydride and diisopropylethylamine (DIPEA)

2:1 in DMF, 45 min each]. The resin was then dried in dichloromethane (DCM) and peptide cleavage was repeated twice (1h+1h) using 50% trifluoroacetic acid (TFA), 47.5% DCM and 2.5% TIS as cleavage mixture, to remove the trityl protections at Gln side chains completely. After cleavage from the resin, the crude peptide was twice decanted from ethyl ether. The crude peptides were purified by means of medium-pressure chromatography on a Biotage Isolera Prime instrument, using reverse phase C18 column and obtained with a purity > 92%. HPLC chromatograms and electron spray ionization high resolution mass spectrometry (ESI-HRMS) spectra for the synthesized peptides are reported in the *Supporting Information.* ESI-HRMS was performed on a Waters Micromass Xevo instrument (Milford, MA).

2.3 Circular dichroism measurements

CD measurements were acquired on a Jasco J-1500 spectrophotometer (Japan), using a 1-mm pathlength quartz cell (Hellma Analytics, Munich, Germany). Methanol or a solution of sodium dodecyl sulfate (SDS) 100mM in milliQ water was used as solvent. The measurements were performed at 25°C, acquiring 16 scans.

2.4 NMR analysis

NMR experiments were performed at 300 K or 297 K with a Bruker AVANCE DRX-400 instrument, operating at the frequency of 400 MHz for ^{1}H. The peptide concentration was 10 mM in CD_3OH for [desAib^{11,12}]pentadecaibin (TABLE 1), or 1 mM for pentadecaibin in 30 mM SDS-d_{12} in H_2O/D_2O 9:1 + 1µL HCl 3 mM in H_2O. Processing and evaluation of the experimental data were carried out using the programs TopSpin and Sparky. CLEAN-TOCSY (spin lock period, 70 ms), ROESY (used for [desAib^{11,12}]pentadecaibin; spin lock period, 100 ms), and NOESY (mixing time, 250 ms) spectra were recorded in the phase-sensitive mode. The -OH signal of CD_3OH and H_2O was suppressed by watergate gradients sequence. All homonuclear 2D spectra were acquired collecting 512 experiments of 48-128 scans each, with a relaxation delay of 1-1.2 s, and 2K data points. The spectral width was 10 ppm in each dimension.

2.5 Leakage experiments

Phosphatidylcholin, phosphatidylglycerol, and phosphatidylethanolamine were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL), while cholesterol was a Merck (Darmstadt, Germany) product. Each lipid mixture was dissolved in $CHCl_3$ in a test tube, dried under N_2, and put under vacuum for two hours. The obtained lipid film was reconstituted with a solution of 5(6)-carboxyfluorescein in 30 mM HEPES buffer (pH 7.4) at room temperature for 1 h. The resulting multilamellar vesicle suspension was sonicated (GEX 400 Ultrasonic Processor, Sigma Aldrich) on ice, twice. The excess of fluorescent dye was eliminated by gel filtration on Sephadex G-75 (Merck). The obtained small unilamellar vesicles (SUVs) were

diluted to a concentration of 0.06 mM with buffer (5 mM HEPES, 100 mM NaCl, pH 7.4). The SUVs were stored at 4 °C and used within 24 h.

The peptide-induced CF leakage from SUVs was measured at room temperature on a Perkin Elmer model MPF-66 spectrofluorimeter. Increasing [peptide]/[lipid] molar ratios (R^{-1}) were obtained by adding increasing aliquots of methanol solutions of the peptide into each cuvette containing 2.5 mL of 0.06 mM SUVs solution. The fluorescence of each cuvette was recorded after 20 min, at 520 nm with λ_{exc} = 488 nm. The percentage of released CF at time *t* was determined as %CF = $(F_t - F_0)/(F_T - F_0) \times 100$, where F_0 is the fluorescence intensity of vesicles in the absence of peptide; F_t, the fluorescence intensity of vesicles at the time *t*, in the presence of peptide, and F_T the total fluorescence intensity determined by disrupting the vesicles through addition of 50 µL of a 10% Triton X-100 aqueous solution.

3. RESULTS AND DISCUSSION

3.1 Peptide synthesis

Pentadecaibin and its short-length analogs (Table 1) were successfully synthesized by manual solid-phase synthesis using *N*,*N'*-diisopropylcarbodiimide (DIC) and Oxyma Pure as activating agents for all but one coupling steps. Indeed, the third residue of the $(Aib)_3$ segment, Aib^{11}, was effectively inserted only by a TFFH-mediated, overnight reaction in the presence of DIEA (a 15-minute preactivation was also needed). The use of other coupling reagents resulted in an incomplete Aib^{11} insertion, even after prolonged and/or repeated couplings. For instance, the oxyma/DIC mediated coupling reaction, repeated three times, failed to achieve quantitative yield: the coupling of Aib^{12} onto Aib^{13} reached a maximum yield of about 90% (estimated by HPLC) while Aib^{11} onto Aib^{12} failed to reach 60% yield even after four cycles of 1-hour coupling.

The yield of this latter difficult step, successful with TFFH, was estimated by HPLC measurement of the amount of the acetylated shorter sequence, obtained from the capping step carried out after the coupling cycle.

All coupling steps involving the other Aib residues were carried out with Oxyma/DIC, but repeated twice. In addition, these couplings were followed by a capping step with acetic anhydride.

After synthesis, the peptides were obtained with fairly good purity, so that purification to >90% could be achieved by means of medium-pressure liquid chromatography alone. HPLC chromatograms and HRMS spectra for the synthesized peptides are reported in the *Supporting Information*.

3.2 Membrane perturbation ability

Pentadecaibin and its shorter analogs were tested for their ability to modify the permeability of small unilamellar vesicles (SUVs) of different compositions, containing the

fluorescent dye 5(6)-carboxyfluorescein (CF). The results are reported in Figure 1. While the native sequence efficiently caused the leakage of CF from both types of SUVs, the performance decreased for the two analogs, in proportion to the number of Aib removed. Indeed, when two out of the three Aib residues in a row of the native sequence are absent, the ability to perturb with model membrane seems to be almost completely lost.

3.3 Conformational analysis

To investigate further on the peculiar behavior of our peptides with model membranes, we performed a conformational analysis in solution by means of circular dichroism (CD) and 2D NMR.

CD measurements were carried out on peptide solutions in (i) methanol and (ii) 100mM aqueous sodium dodecyl sulfate (SDS), which forms micelles. The results are reported in Figure 2. All peptides adopt a well-developed helical conformation in methanol solution. Their ratios *R* between the molar ellipticities at the two negative maxima (Table 2) in methanol point towards a prevalence of α-helix over the 3_{10}-helix, as it is common for medium- and long-length peptaibols. Indeed, the *R* value of about 0.9 is quite close to 1, which is the value associate to a fully α-helical structure [11]. *R* values greater than 1, as those observed in SDS micelles (Table 2), can be associated with the presence of helical aggregates [12]. The formation of helical bundles in membranes is often a prerequisite for the bioactivity of peptaibols. Pentadecaibin and its shorter analogs may thus adopt right-handed α-helical conformations in solution, and their helices self-associate in aggregates in the presence of micelles.

As already pointed out, the onset of self-assembly modifies the peptide CD profile, and may hide some information on its 3D-structure. To further investigate on the peptide helical conformation and evaluate its stability, we performed a 2D-NMR analysis on the native pentadecaibin in the presence of micelles of SDS-d_{12}. As expected, the NOESY spectrum unambiguously confirmed the presence of an α-helical conformation. The two, most informative portions of the spectrum, namely the amide proton and the *fingerprint* regions, are reported in the *Supporting information*.

As the peptides did not show any clear-cut difference on their 3D-structure in solution, we next decided to investigate by NMR the shortest analog, [desAib^{11,12}]pentadecaibin, because it displayed the lowest ability to perturb our model membranes. To assess the stability of its helical conformation, without possible influence of aggregation phenomena, we recorded its 2D NMR spectra in methanol.

The two, most informative regions of the ROESY spectrum are reported in FIGURES 3 and 4. Several, long-range cross-peaks were detected, which are diagnostic of a fully-developed helical conformation. In particular, the presence of a long-range, $\alpha_i \rightarrow NH_{i+4}$ correlation confirms the presence of an α-helix.

We conclude that the loss of leakage activity observed for the shortest analog cannot be attributed to the unraveling of the helical conformation, that conversely seems to be maintained.

3.4 Peptide amphiphilicity

The loss of activity can be due to several reasons, such as variations in peptide/membrane binding, peptide aggregation in water, or ability to attain a transmembrane orientation. To shed light on the issue of amphipathicity, often relevant for membrane interactions, we drew models of the three peptides as full α-helices (reported in the *Supporting information*). At first, we compared their length with the thickness of the model membranes used, but we did not find relevant differences. The length of the three peptides (from the first to the last NH) is 21.47 Å, 20.13 Å, and 18.53 Å, for pentadecaibin, [desAib^{12}]pentadecaibin, and [desAib^{11,12}]pentadecaibin, respectively. The overall bilayer thickness of a model membrane is about 48 Å [13], while the hydrophobic core is about 28 Å thick [12]. Therefore, the difference in peptide lengths is not the main reason for the different performances in the leakage assay.

We thus focused on the amphipathicity of the helical structures, since it is one of the features which mostly affect membrane activity [14]. The view along the helix axis for the three model structures (FIGURE 5) shows a clear perturbation of the (modest) amphipathic character when the C-terminal Aibs are removed. Indeed, the two hydrophilic residues of Gln, that are on the same face of the helix in the natural sequence, result opposite to each other once two Aib residues are removed from the C-terminus. In parallel, the hydrophobic face is affected by the lack of two Aib residues: the hydrophobic residue Phol^{15}, that falls very close to Leu^{4} and Val^{8} in the native pentadecaibin sequence, is placed further away in [desAib^{12}]pentadecaibin and even between the two hydrophilic Gln residues in [desAib^{11,12}]pentadecaibin.

The importance of each Aib residue in the peptide sequences was also assessed. Aibs at positions 1 and 5 are placed at one helix turn distance, thus supporting the onset of the conformation. Interestingly, the presence of Aib residues at those two positions is a common feature of medium- and long-length peptaibols, such as alamethicins and trichobrachins [1]. Similarly, Aib residues at position 7 and 9 contribute to the structure stabilization. Aibs at those two positions are present in the sequence of medium-length peptaibols such as zervamicin [1] and some cephaibols [15].

4. CONCLUSIONS

In this contribution we propose an effective procedure for the total synthesis of the medium-length peptaibol pentadecaibin, and two analogs thereof. The devised solid-phase procedure was successful, despite the presence of as much as 50% Aib residues, three of which in a row. By a 24 h-reaction mediated by TFFH (3 eq. of the incoming residue) we successfully obtained quantitative yield on the formation of the most difficult peptide bond, namely Aib over the Aib-Aib dipeptide. The method can most likely be extended also to the production of analogs containing sterically-hindered, spin-labeled amino acid residues, such as 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) suitable for

spectroscopic studies on the mechanism of peptide-membrane interaction. Aib to Ala mutants can also be synthesized, to further study the effects of Aib removal.

Our leakage experiments showed that the shortest analog did not retain the ability of pentadecaibin to perturb the membrane permeability, while the spectroscopic studies confirmed the presence of a well-defined helical structure even for that shortest peptide. Therefore, the difference in the membrane interactions cannot be ascribed to loss of helical content. Rather, since the absence of two Aib residues at the peptide C-terminus perturbs the overall amphiphilic character of the helix, we are inclined to attribute the loss of membrane activity to the decrease of peptide amphiphilicity. The peculiar correlation between peptide sequence and leakage activity of the peptides herein described may be exploited to further investigate the role played by amphipathicity on peptide self-assembly and membrane interaction.

ACKNOWLEDGMENTS

M.D.Z. and F.F. are grateful to the Italian Ministry for University and Research (MIUR) (PRIN Project 2020833Y75 and 20173LBZM2) and to the University of Padova (Project P-DiSC#04BIRD2019-UNIPD and Uni-Impresa 2020-UNPD0Z9-0069266) for funding. D.S.H. acknowledges the support of a COIMBRA fellowship.

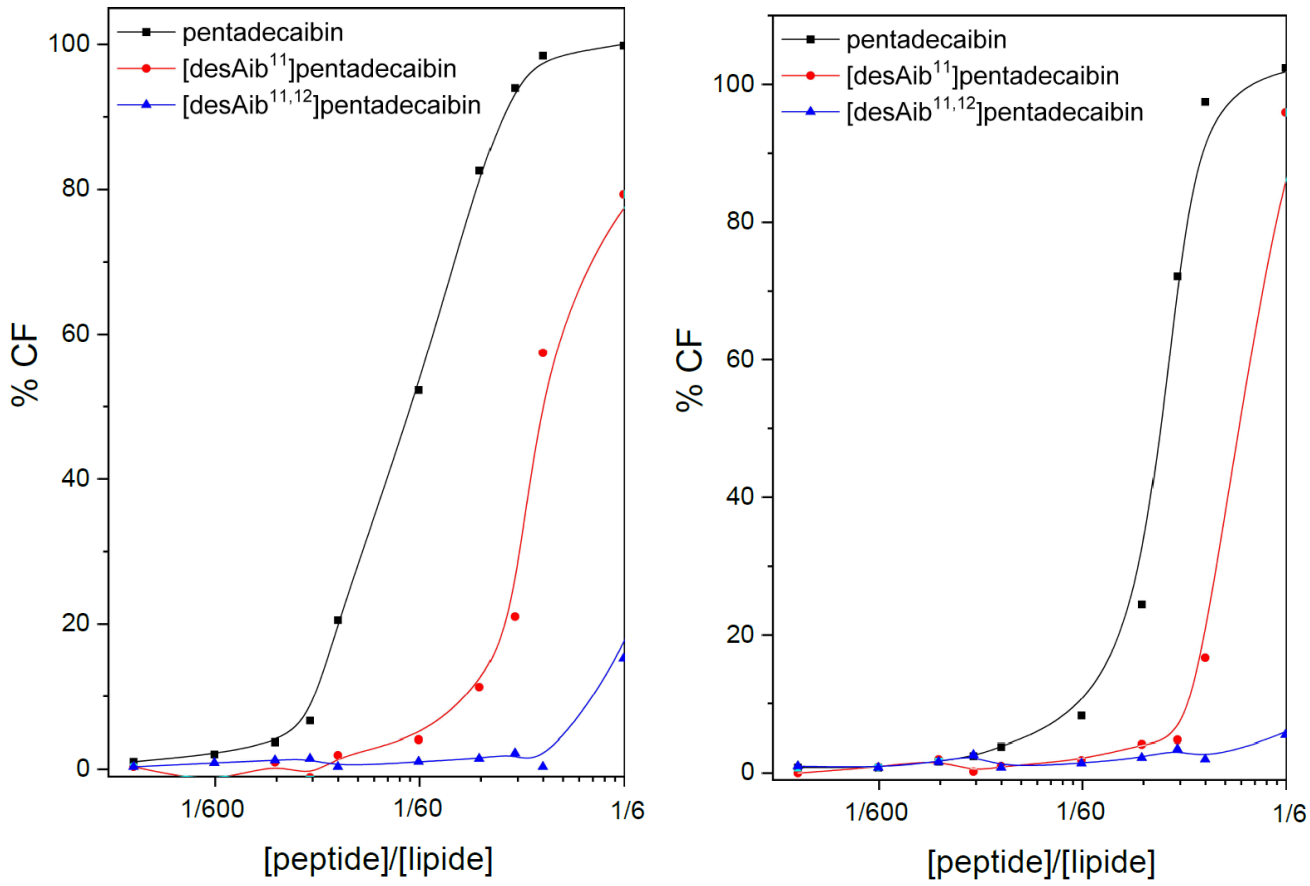


FIGURE 1. CF leakage induced by pentadecaibin and its shorter analogs on phosphatidylcholine/cholesterol 7:3 (left) and on phosphatidylglycerol/phosphatidylethanolamine 7:3 (right) SUVs.

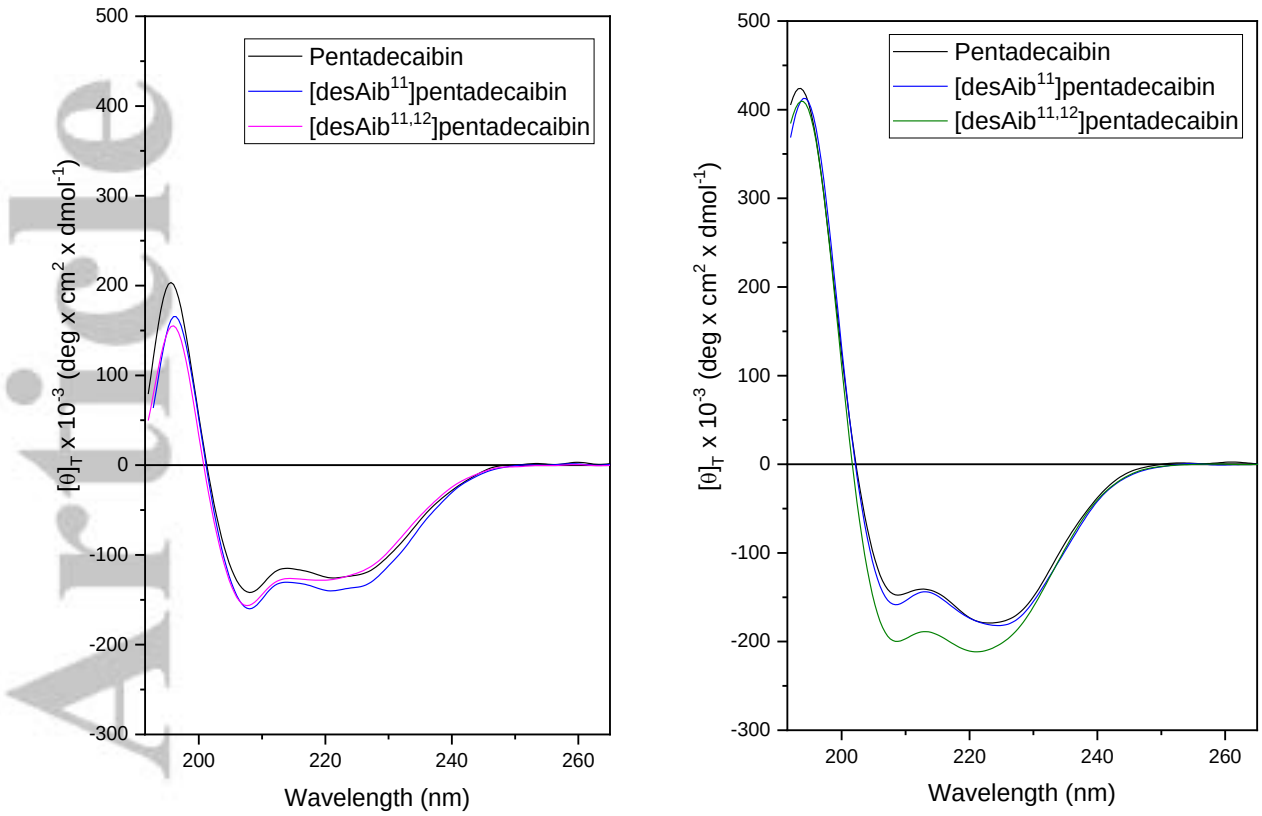


FIGURE 2. Overlapping of the CD profiles of pentadecaibin and its shorter analogs in methanol (left) and in micelles of SDS 100 mM in water (right). Peptide concentration: 0.1 mM.

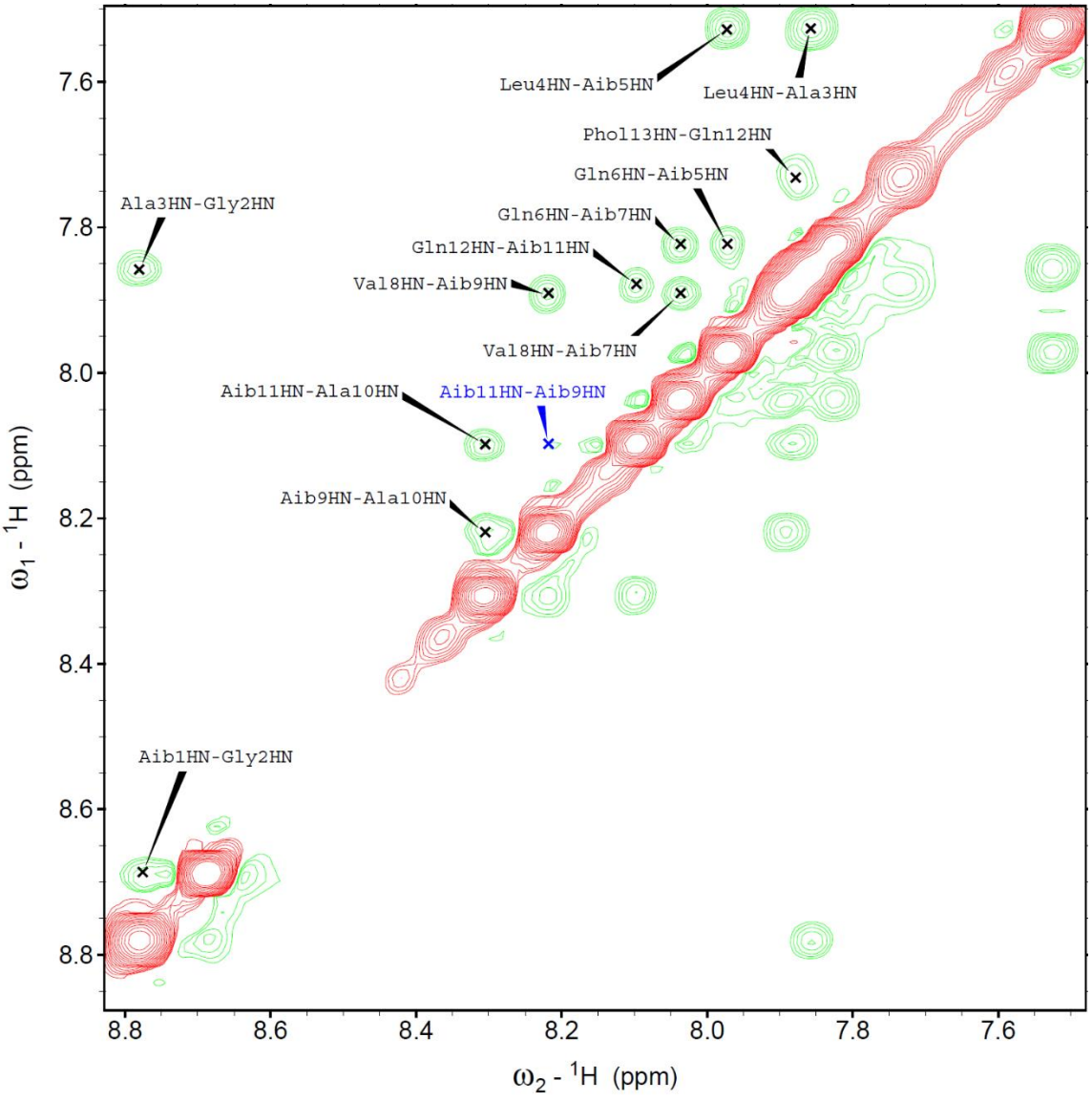


FIGURE 3. Amide region of the ROESY spectrum acquired for [desAib^{11,12}]pentadecaibin in CD_3OH (10 mM, 300 K, 400 MHz). A long-range cross-peak $NH_i \rightarrow NH_{i+2}$, diagnostic of a well-developed helical conformation, is highlighted in blue.

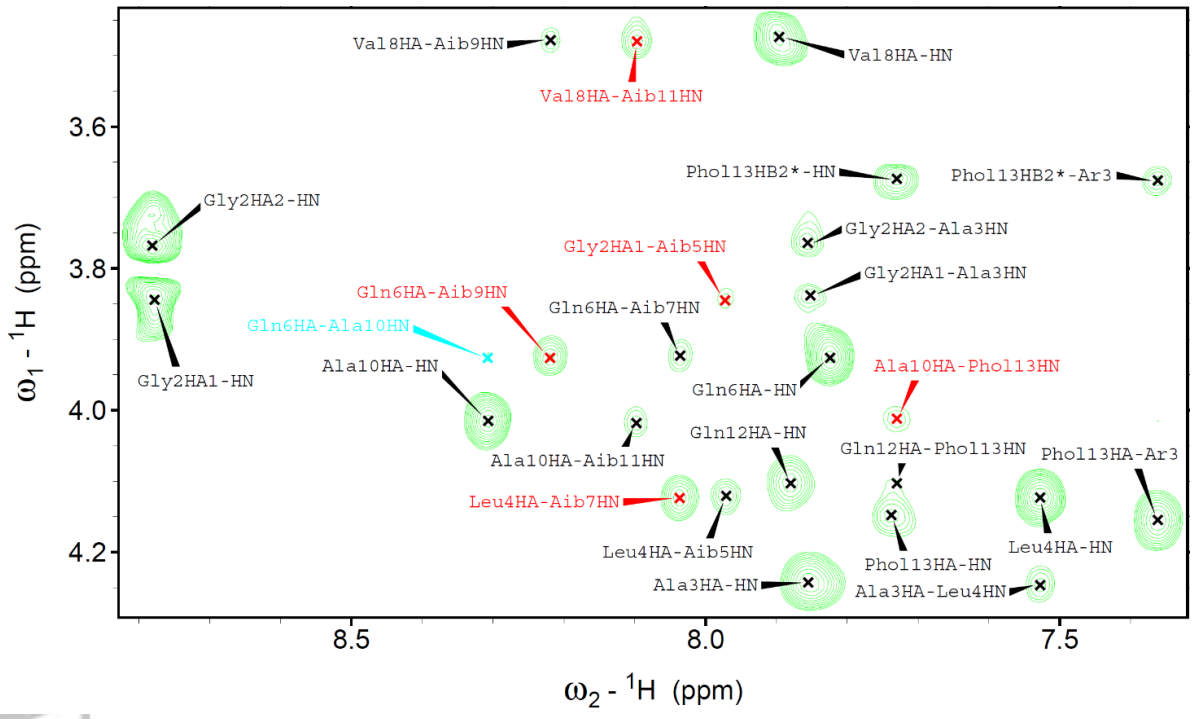


FIGURE 4. *fingerprint* region of the ROESY spectrum acquired for [desAib^{11,12}]pentadecaibin in CD_3OH (10 mM, 300 K, 400 MHz). Long-range cross-peaks $^{\alpha}H_i \rightarrow NH_{i+3}$, diagnostic of a well-developed helical conformation, are highlighted in red, while one $^{\alpha}H_i \rightarrow NH_{i+4}$ correlation, diagnostic of the presence of a α-helix, is highlighted in cyan.

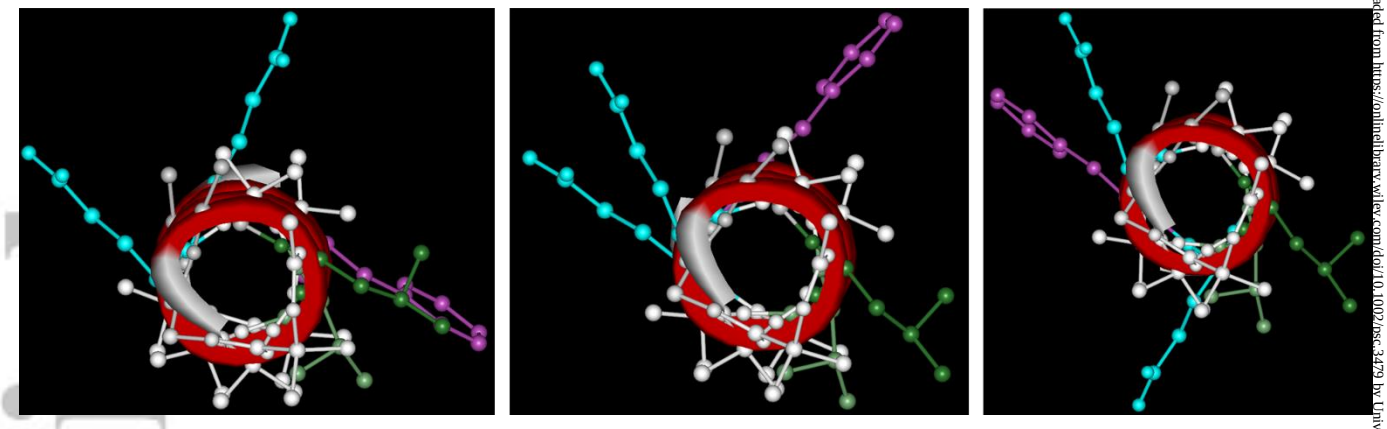


FIGURE 5. View along the helix axis for the model structures drawn for pentadecaibin (left) [desAib^{11}]pentadecaibin (middle), and [desAib^{11,12}]pentadecaibin (right). The side chain of Gln residues is highlighted in cyan, while Phol and Leu side chains are magenta and dark green, respectively.

TABLE 1. Primary structure of pentadecaibin and its short-length analogs synthesized in this work

No.	Peptide name	Sequence
1	pentadecaibin	Ac-Aib[1]-Gly-Ala-Leu-Aib[5]-Gln-Aib-Val-Aib-Ala[10]-Aib-Aib-Aib-Gln-Phol[15]
2	[desAib[11]]pentadecaibin	Ac-Aib[1]-Gly-Ala-Leu-Aib[5]-Gln-Aib-Val-Aib-Ala[10]-Aib-Aib-Gln-Phol
3	[desAib[11,12]]pentadecaibin	Ac-Aib[1]-Gly-Ala-Leu-Aib[5]-Gln-Aib-Val-Aib-Ala[10]-Aib-Gln-Phol

TABLE 2. *R* values obtained from the CD spectra for the three peptides.

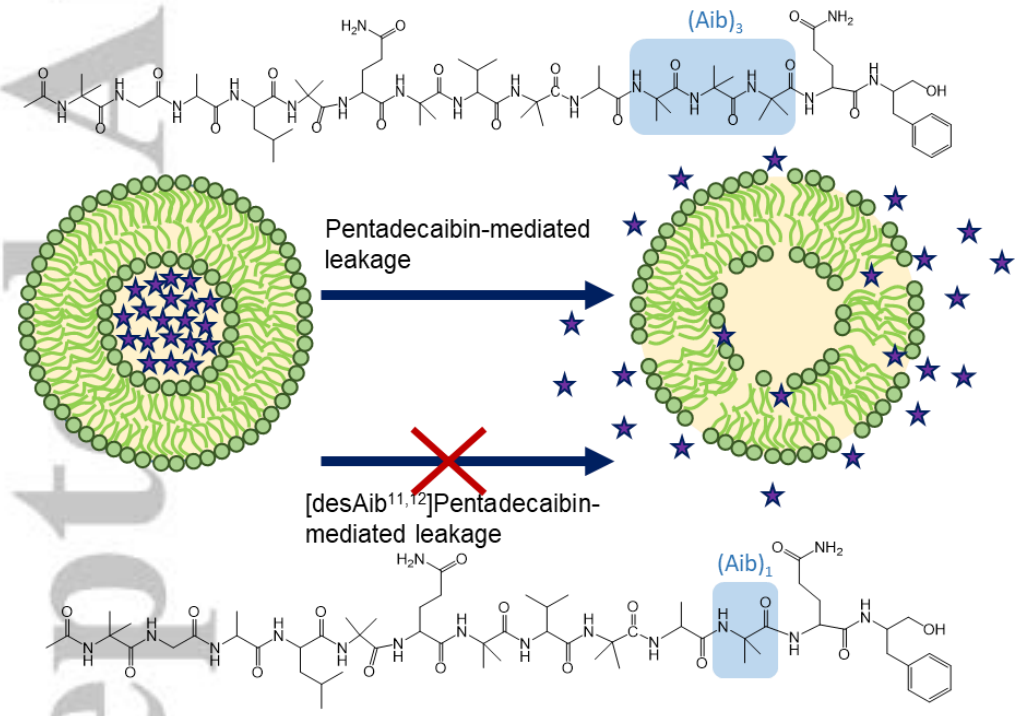
Peptide	R [θ_{222}]/[θ_{208}] in MeOH	R [θ_{222}]/[θ_{208}] in SDS 100mM
Pentadecaibin	(-125.5)/(-141.8) = 0.88	(-179)/(-147) = 1.2
[desAib[11]]pentadecaibin	(-139.5)/(-160) = 0.87	(-182)/(-158) = 1.15
[desAib[11,12]]pentadecaibin	(-126.5)/(-156.5) = 0.81	(-211.6)/(-200) = 1.06

((Graphical Abstract))

Total synthesis of the natural, medium-length, peptaibol pentadecaibin and study of the chemical features responsible for its membrane activity

*Laura Morbiato, David S. Haneen, Fernando Formaggio, Marta De Zotti**

Pentadecaibin, a natural medium-length peptaibol, does not comprise any hydroxyprolines or prolines, and its helix is not kinked. An effective synthesis procedure is described to produce pentadecaibin and also two shorter analogs. The membrane activity of pentadecaibin heavily depends on the presence of three consecutive α-aminoisobutyric acid residues.



REFERENCES

1 Toniolo C, Brückner H. Peptaibiotics: Fungal Peptides Containing α-Dialkyl α-Amino Acids. Verlag Helvetica Chimica Acta: Zürich, Switzerland and Wiley-VCH: Weinheim, Germany, 2009.

2 De Zotti M, Biondi B, Formaggio F, Toniolo C, Stella L, Park Y, Hahm K-S. Trichogin GA IV: an antibacterial and protease-resistant peptide. *J. Pept. Sci*. 2009; **15**: 615‑619.

3 Milov AD, Tsvetkov Yu D, Raap J., De Zotti M, Formaggio F, Toniolo C, Conformation, self-aggregation, and membrane interaction of peptaibols as studied by pulsed electron double resonance (PELDOR) spectroscopy, *Biopolymers (Pept. Sci.)* 2016; **106**: 6-24.

4 Shai Y. Mode of action of membrane active antimicrobial peptides. *Biopolymers* 2002; **66**: 236-248.

5 Peggion C, Formaggio F, Crisma M, Epand RF, Epand RM, Toniolo C. Trichogin: a paradigm for lipopeptaibols. *J. Pept. Sci.* 2003; **9**:679–689.

6 Fringeli UP, Fringeli M. Pore formation in lipid membranes by alamethicin. *Proc Natl Acad Sci U S A*. 1979; **76**:3852-3856.

7 De Zotti M, Biondi B, Peggion C, Park Y, Hahm K-S, Formaggio F, Toniolo C. Synthesis, preferred conformation, protease stability, and membrane activity of heptaibin, a medium-length peptaibiotic, *J. Pept. Sci.* 2011; **17**: 585-594.

8 Bortolus M, Dalzini A, Formaggio F, Toniolo C, Gobbo M, Maniero AL. *Phys. Chem. Chem. Phys.* 2016;**18**: 749-760

9 Hou X, Sun R, Feng Y, Zhang R, Zhu T, Che Q, Zhang G, Li D. Peptaibols: Diversity, bioactivity, and biosynthesis. *Eng. Microbiol*. 2022; **2**: 100026.

10 van Bohemen A-I, Ruiz N, Zalouk-Vergnoux A, Michaud A, du Pont TR, Druzhinina I, Atanasova L, Prado S, Bodo B, Meslet-Cladiere L, Cochereau B, Bastide F, Maslard C, Marchi M, Guillemette T, and Pouchus YF. Pentadecaibins I–V: 15-Residue Peptaibols Produced by a Marine-Derived Trichoderma sp. of the Harzianum Clade. *J. Nat. Prod.* 2021; **84**: 1271-1282.

11 Manning MC, Woody RW. Theoretical CD studies of polypeptide helices: examination of important electronic and geometric factors. *Biopolymers* 1991; **31**: 569-585.

12 Wallimann P, Kennedy RJ, Kemp DS. Large circular dichroism ellipticities for N-templated helical polypeptides are inconsistent with currently accepted helicity algorithms. *Angew. Chem. Int. Edit*. 1999; **38**: 1290-1292.

13 Bobone S, Gerelli Y, De Zotti M, Bocchinfuso G, Farrotti A, Orioni B, Sebastiani F, Latter E, Penfold J, Senesi R, Formaggio F, Palleschi A, Toniolo C, Fragneto G, Stella L. Membrane thickness and the mechanism of action of the short peptaibol trichogin GA IV. *Biochim. Biophys. Acta (Biomembranes)* 2013; **1828**:1013-1024.

14 Yeaman MR, Yount YN. Mechanisms of Antimicrobial Peptide Action and Resistance, *Pharm. Rev*. 2003; **55**:27-55.

15 Schiell M, Hofmann J, Kurz M, Schmidt FR, Vériest L, Vogel M, Wink J, Seibert G. Cephaibols, New Peptaibol Antibiotics with Anthelmintic Properties from Acremonium tubakii DSM 12774, *J. Antibiot.* 2001; **54**: 220-233.