Special Collection

Hybrid Peptides Based on α-Aminoxy Acids as Antimicrobial and Anticancer Foldamers

Laura Sinatra,[a] Lisa Kolano,[b, c] Maik Icker,[d] Stefan R. Fritzsche,[a] Daniela Volke,[b, c] Ines Gockel,[e] René Thieme,[e] Ralf Hoffmann,[b, c] and Finn K. Hansen*[f]

α-Aminoxy peptides represent an interesting group of peptidomimetics with high proteolytic stability and the ability to fold into specific, predictable secondary structures. Here, we present a series of hybrid peptides consisting of α-aminoxy acids and α-amino acids with cationic and aromatic, hydrophobic side chains in an alternating manner synthesized using an efficient protocol that combines solution- and solid-phase synthesis. 2D ROESY experiments with a representative hexamer suggested the presence of a 7/8 helical conformation in solution. Biological evaluation revealed a significant impact of the peptide chain length and the *N*-terminal cap on the antimicrobial and anticancer properties of this series of hybrid peptides. The Fmoc-capped peptide **6e** displayed the most potent antimicrobial activity against a panel of Gram-negative and Gram-positive bacterial strains (e.g. against *E. Coli*: MIC = 8 mg/L; *S. aureus*: MIC = 4 mg/L).

Introduction

A great number of lead structures and modes of action utilized in modern medicinal chemistry are inspired by nature. The rising complexity of human diseases has shifted the focus of research in drug development from standard small molecules following Lipinski's rule of five to structural more complex approaches, e.g. antibody-drug conjugates, macrocycles or proteolysis-targeting chimeras (PROTACs).[1] Naturally occurring peptides hold crucial functions in human physiology in their roles as enzyme inhibitors, neurotransmitters, growth factors, ion channel ligands or anti-infective compounds.[2] However, their targeted use as therapeutics, although they are often highly selective, effective, mostly safe and well tolerated, is still limited due to their weaknesses. The typical drawbacks of peptide-based drug candidates include limited bioavailability, tendency for aggregation and poor stability towards hydrolysis and oxidation.[3] Foldamers, defined by Gellman[4] as synthetic polymers with a strong tendency to fold into specific, compact conformations, are an interesting and promising class of molecules with the advantage of adjustable physical, chemical and biological properties to meet the common challenges of peptide-based drugs.[4,5] Remarkable efforts have been made during the past years in the investigation of new backbones, which are able to form well defined secondary structures apart from the three classical naturally occurring backbones (proteins, ribonucleic acids and polysaccharides).[6] In the field of peptidomimetics, among others, two important classes have emerged as promising candidates for foldamers: the ß-peptides investigated by Seebach and Gellman as pioneer researchers[7–9] and α-aminoxy peptides, the oxa-analogues of ß-peptides, which were intensively investigated by the group of Yang.[10,11]

The use of α-aminoxy acids as building blocks for oligomers brings significant advantages. On the one hand, the aminoxy amide bond is considerably more stable to enzymatic degradation.[12] On the other hand, peptides comprising α-aminoxy acids form predictable and controllable secondary structures due to the more rigid backbone resulting of the repulsion between the respective lone pairs of the heteroatoms.[13] The unique folding behavior of α-aminoxy peptides is induced by the formation of a so-called α-N–O-turn, a rigid eight membered ring with strong intramolecular hydrogen bonds between the C=O_i as acceptor and the amide in i + 2 position (N–H_{i+2}) as proton donator, which was discovered by Yang and coworkers.[14] By solving an X-Ray crystal structure of

[a] L. Sinatra, S. R. Fritzsche
Institute for Drug Discovery, Medical Faculty
Leipzig University
Brüderstr. 34, 04103 Leipzig (Germany)
[b] L. Kolano, Dr. D. Volke, Prof. R. Hoffmann
Institute of Bioanalytical Chemistry
Faculty of Chemistry and Mineralogy
Leipzig University
Deutscher Platz 5, 04103 Leipzig (Germany)
[c] L. Kolano, Dr. D. Volke, Prof. R. Hoffmann
Center for Biotechnology and Biomedicine (BBZ)
Deutscher Platz 5, 04103 Leipzig (Germany)
[d] Dr. M. Icker
Institute of Organic Chemistry
Leipzig University
Johannisallee 29, 04103 Leipzig (Germany)
[e] Prof. Dr. I. Gockel, Dr. R. Thieme
Department of Visceral, Transplant
Thoracic and Vascular Surgery
University Hospital of Leipzig
Liebigstraße 20, 04103 Leipzig (Germany)
[f] Prof. Dr. F. K. Hansen
Department of Pharmaceutical and Cell Biological Chemistry
Pharmaceutical Institute
University of Bonn
An der Immenburg 4, 53121 Bonn (Germany)
E-mail: finn.hansen@uni-bonn.de

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cplu.202000812

This article is part of a Special Collection on "Synthesis, Properties, and Applications of Foldamers".

an α-aminoxy hexapeptide, we demonstrated that α-aminoxy peptides can fold into a 2_8-helical conformation with precisely two residues per turn and a helical pitch of 5.8 Å.[7] Furthermore, the X-Ray crystal structure confirmed the presence of repetitive α-N–O-turns. Of note, the 2_8-helix is capable of mimicking the spatial arrangement of peptide side chains in α-helices and 3_{10}-helices.[7,15]

Heterochiral α-peptide/α-aminoxy peptide hybrids, peptides with alternating amide and aminoxy amide bonds, form another unique type of helix, namely the 7/8 helix with iterative α-N–O-turns and γ-turns, a seven membered ring formed by an intramolecular hydrogen bond between $C{=}O_i$ and NH_{i+2}.[16] Their rigid backbone and their preference to adopt helical conformations make them interesting candidates for the development of new antimicrobial peptides (AMPs). Most AMPs are cationic, amphiphilic molecules with opposing lipophilic and polycationic faces. Their amino acid composition, amphipathicity, cationic charge and size allow them to attach and insert into membrane bilayers.[17,18] The negative surface charge of bacterial cells is a characteristic also shared by cancer cells. Based on this fact it is not surprising that some AMPs, but not all, are active against cancer cells and anticancer peptides (ACPs) have emerged as a potential alternative approach for cancer therapy.[18] The common initial mode of action of AMPs and ACPs is the electrostatic interaction with the negatively charged cell membrane,[18,19] followed by a membrane-disruptive mechanism, e.g. through pore (barrel stave and toroidal pore models) or micelle formation (carpet model), or a cell penetration mechanism (Figure 1).

The use of α-aminoxy oligopeptides as anticancer foldamers has already been thoroughly investigated by our group.[7] In this work we present the combined solution- and solid-phase synthesis and conformational studies of α-peptide/α-aminoxy peptide hybrids as potential AMPs and ACPs.

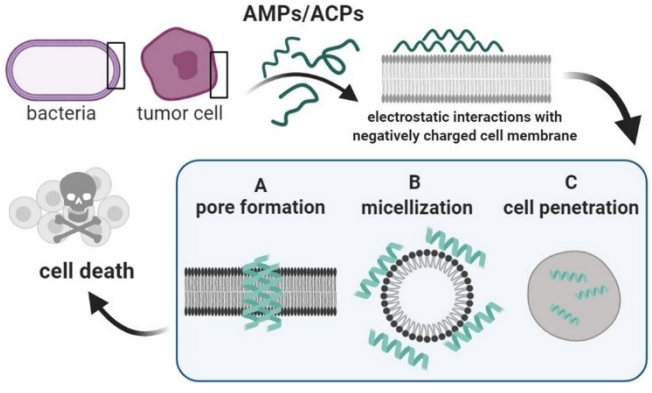
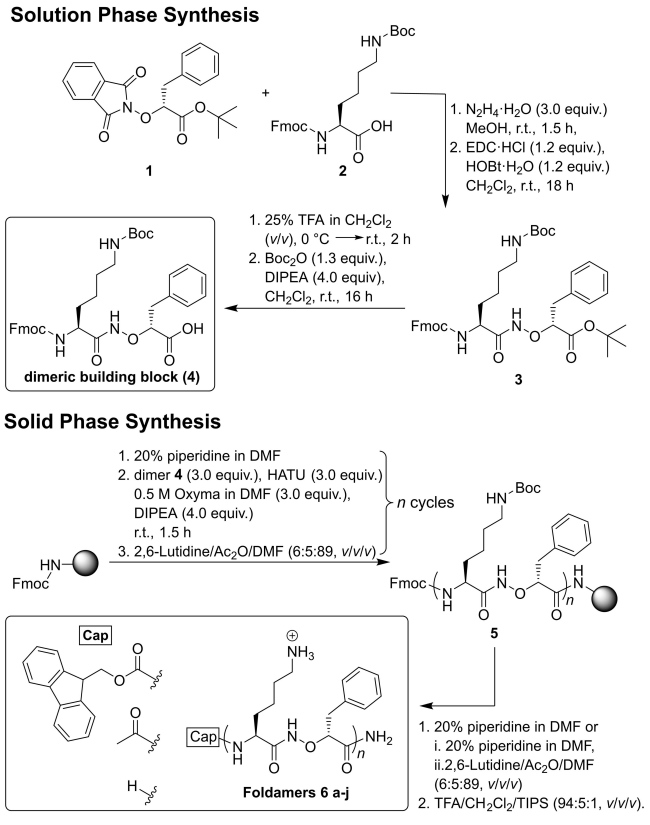


Figure 1. Common initial mode of action of AMPs/ACPs and selected mechanisms.

Results and Discussion

Chemistry

For synthesis of the peptidomimetics we chose lysine residues as cationic component and phenylalanine residues as hydrophobic component in an alternating manner and equal proportions. The monomeric building block Phth-D-^{NO}Phe-O*t*Bu **1** was synthesized from the respective H_2N-L-Phe-OH according to literature known procedures.[7] For oligomer synthesis we decided to use a combined solution- and solid-phase synthesis strategy, where Fmoc-NH-L-Lys(Boc)-D-^{NO}Phe-OH (**4**) was prepared in solution phase and afterwards used as dimeric building block for the subsequent solid phase synthesis of the peptides. This strategy features several benefits: 1.) By using the dimer as building block we can utilize the simple and straightforward Fmoc-strategy for the solid phase synthesis, 2.) less coupling cycles are needed and 3.) the more critical formation of the aminoxy amide bond is performed gently under milder and less concentrated conditions in solution with excellent yields up to 96%. In detail, the phthaloyl group in **1** was deprotected using hydrazine monohydrate, followed by an EDC·HCl/HOBt·H_2O mediated amide coupling with Fmoc-L-Lys(Boc)–OH (**2**) at a low reaction concentration of <0.1 M to afford the *t*-Bu-protected dimer **3** (Scheme 1). During the following deprotection of the *tert*-butyl ester using acidic conditions, the Boc-group of the lysine residue was simultaneously deprotected. The final Boc-protection of the lysine side chain afforded the free dimer **4**,



Scheme 1. Combined solution and solid phase synthesis of oligomers **6a**–**6j**.

ready to use in the subsequent solid phase synthesis. First attempts for the amide coupling on solid support using commercially available Rink amide resin were performed using Oxyma/DIC as coupling system. Test cleavage of the first hybrid peptides as well as the TNBS-test indicated that the couplings were not completed. Thus, we decided to change the coupling system to Oxyma/HATU/DIPEA leading to complete amide formation within 90 min.

We synthesized a library of foldamers containing eight hybrid peptides (**6a**–**d** and **6g**–**j**) with different chain lengths (n = 1–8). Due to the easier purification, we decided to synthesize the *N*-terminal acetylated foldamers. In addition, we synthesized decamers, where we introduced different modifications. On the one hand, we synthesized three decamers with different *N*-terminal caps, including Fmoc (**6e**) acetyl (**6g**), and capless (free *N*-terminus, **6f**) to investigate the impact of the *N*-terminal position on the biological activity of the foldamers. On the other hand, we synthesized a decamer (peptide **7**) containing only natural amino acids to study its stability in comparison to the hybrid peptides. Generally, a Rink amide resin was chosen for the synthesis of the peptides. However, in the case of **6b** a Sieber amide resin was used due to degradation and low purity of the tetramer when using the rink amide cleavage cocktail. After cleavage of the peptides with the respective cleavage solution the crude peptides were purified by preparative or semipreparative RP-HPLC to a purity > 95%. The fractions containing the desired peptides were lyophilized and characterized by HR-MS.

Solubility

We investigated the solubility of peptides **6c**, **6d** and **6h** in different aqueous and non-aqueous solvents (Table 1). All peptides showed excellent solubility in aqueous environment with solubilities > 10 mM. Furthermore, all peptides showed high solubility in DMSO and MeOH. However, in less polar solvents the solubility is rapidly declining. Interestingly, the solubility of the peptides in MeCN decreases with increasing chain length, while the solubility in CH_2Cl_2 is improved with increasing peptide length. In addition, no tendency to aggregate was observed.

Table 1. Solubilities [mM] of hybrid peptides **6c, 6d** and **6h** in the respective solvent after centrifugation.[a]

Solvent	Peptide solubility [mM][b]		
	6c	**6d**	**6h**
H_2O	> 10	> 10	> 10
Tris buffer (10 mM, pH 7.5)	> 10	> 10	> 10
CHES buffer (10 mM, pH 9.5)	> 10	> 10	> 10
MeCN	0.82 ± 0.02	0.12 ± 0.06	< 0.01
20% MeCN in H_2O (*v*/*v*)	> 10	> 10	> 10
DMSO	> 10	> 10	> 10
MeOH	> 10	> 10	> 10
CH_2Cl_2	0.10 ± 0.08	0.27 ± 0.10	0.47 ± 0.24

[a] Concentrations were determined in duplicate series by HPLC analysis. [b] > 10 mM: samples were completely dissolved in respective solvents at 10 mM.

Stability

To evaluate the hybrid backbone comprising of alternating amide and aminoxy amide bonds as potential peptidomimetics we studied its stability against enzymatic degradation. For the *in vitro* evaluation, we chose the enzyme trypsin, a serine protease with the function to cleave amide bonds subsequent to positively charged amino acids, e.g. Lys, Arg. To study the impact of the modified backbone contrary to the classical peptide backbone, we investigated the stability of the hybrid peptide **6g** and its α-peptide analogue **7** (Figure 2A) consisting of only amide bonds. Figure 2B shows that peptide **7** was degraded completely in less than two hours, whereas only 37% of hybrid peptide **6g** was degraded during the same time (Figure 2B). Furthermore, control experiments without trypsin demonstrated a high chemical stability for both peptides within 72 h (Figure 2C), suggesting that the degradation observed in Figure 2B comes from enzymatic instability and not from chemical instability in buffer. To conclude, these experiments demonstrate that the hybrid backbone is significantly more stable against enzymatic degradation than the common peptide backbone and justify to study the folding propensities and biological activities of α-peptide/α-aminoxy peptide hybrids in more detail.

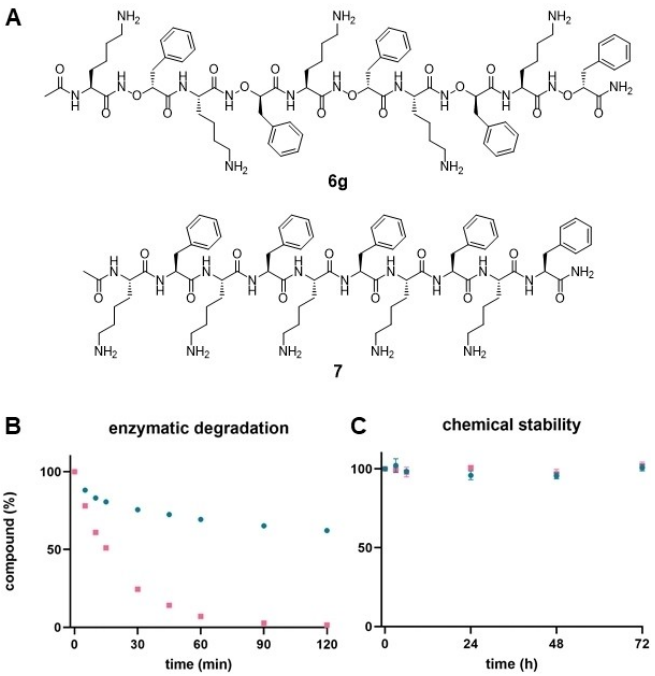


Figure 2. A) Decamer **6g** comprising of alternating aminoxy and amino acids and its non-hybrid counterpart **7**. B) Stability of peptides **6g** (●) and **7** (■) towards enzymatic degradation by trypsin (0.02 mg/mL, 10 mM Tris buffer pH 7.5 at 37 °C) determined in triplicate series via HPLC analysis. C) Chemical stability of the peptides **6g** (●) and **7** (■) in 10 mM Tris buffer (pH 7.5) at 37 °C determined in triplicate series.

Conformational Studies

The biological activity of antimicrobial/anticancer peptides and their peptidomimetic analogues are strongly associated with their conformational folding. The secondary structure of the hybrid peptides in solution was therefore studied in detail by NMR and CD spectroscopy. Based on previous reports by Yang and co-workers, we expected the backbone of the oligomers to align in alternating seven- and eight-membered rings through formation of intramolecular hydrogen bonds and thus to form a helical secondary structure.[14,16] In order to probe this hypothesis, we selected hexamer **6c** as representative example to investigate its secondary structure by 2D NMR, i.e. ROESY (Rotating frame Overhauser Effect SpectroscopY). NMR signal assignments through COSY, HSQC and HMBC methods verified the common appearance of the aminoxy amide protons in the region of 10.5 to 11.5 ppm, whereas the lysine amide protons resonate between 7.6 and 8.6 ppm; all NMR signals of the backbone are distinguishable and have been fully assigned. It turned out that the most promising ROESY data is acquired at −5 °C as the aminoxy protons tend to exchange too fast and hence show barely any ROE peak at higher temperatures. For solubility reasons we added some small portions of methanol-d_3 and $CDCl_3$ to a 6.2 mM solution of **6c** in acetonitrile-d_3, as acetonitrile should not inhibit the formation of intramolecular hydrogen bonds.

The ROESY spectrum (Figure S1, Supporting Information) revealed a clear and repetitive correlation pattern, which, at first glance, indicates some ordering of specific 'units'. Figure 3 summarizes all ROE correlation signals observed for the hexamer. Starting at the *N*-terminus, the Ac–NH proton H_a shows a ROE correlation to the following NH proton in the sequence (H_b), which is an aminoxy proton. This kind of connectivity is only plausible in case there exists a hydrogen bond between the (acidic) aminoxy proton H_b (NH_{i+2}) and the carbonyl oxygen ($C{=}O_i$) of the acetyl group to form a seven-membered ring. All amide protons of the lysine residues within the hexamer exhibit similar correlations.

The Lys-amide protons of the second (H_c) and third unit (H_e) within the hexamer also possess spatial proximity to the respective C_α proton (H_2 and H_4) of the ^{NO}Phe residue ($C_\alpha H_{i-1}$) as indicated by a strong ROE peak. The latter finding provides strong evidence of an eight-membered ring being formed as the system establishes a hydrogen bond between the carbonyl oxygen ($C{=}O_i$) of one lysine and the amide proton (NH_{i+2}) of the next lysine residue within the sequence. Even proton $H_{g'}$ of the C-terminal amide group seems fixed in a hydrogen bond (ring closure with $C{=}O_{i-2}$) because it features an intense ROE correlation to the C_α proton H_6 of the preceding ^{NO}Phe. Each Lys amide proton (NH_i) furthermore give ROEs to its C_α proton ($C_\alpha H_i$); the $C_\alpha H$ on the other hand reveals a correlation signal to each aminoxy NH of the following ^{NO}Phe.

All above-mentioned ROE connectivities are repetitively found for every ring unit of the hexamer. This kind of periodic pattern is in agreement with the findings from Li and Yang[16] who describe 7/8 helical conformation as a result of α-N–O turns and γ-turns being formed in mixed peptides of alternating aminoxy and amino acids. We therefore conclude that the conformation of the representative hexamer **6c** appears helically ordered through alternating seven- and eight-membered rings.

CD-spectra from oligomers **6c**, **6d** and **6h**, recorded at room temperature in 2,2,2-trifluoroethanol (TFE) are shown in Figure 4A. All three compounds showed consistent CD-curves with a minimum between 225 nm and 230 nm and a zero-crossing between 217 nm and 219 nm. These spectra, however, showed an altered CD-curve in comparison to previously published CD-spectra of heterochiral hybrid peptides composed of alternating α-aminoxy and α-amino acids containing only aliphatic residues.[20] Since our results from the 2D NMR studies suggested a folding into a 7/8 helical conformation, it can be assumed that the cationic and aromatic residues utilized in this study have a significant effect on the CD curve. Since α-helical AMPs are usually disordered and do not fold in aqueous environment, we further investigated the structural behavior of the hybrid peptides in aqueous solution.[21] In addition, we measured CD-spectra of these peptides in 60% aqueous TFE solution (Figure 4B). This solvent combination is often used for conformational studies of α-peptides as model membrane system, because it is assumed that this mixture mimics the hydrophobic environment of cellular membranes.[22,23] Based on

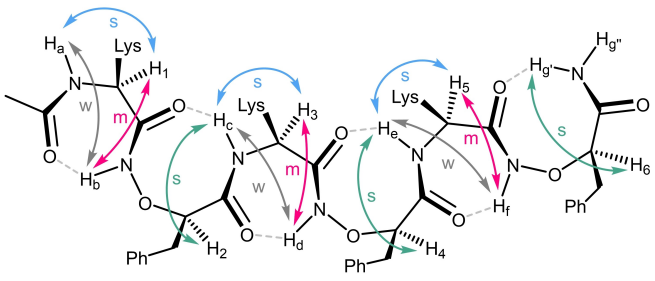


Figure 3. Summary of ROE connectivity observed for hexamer **6c** at −5 °C (6.2 mM in a mixture of acetonitrile-d_3/methanol-d_3/$CDCl_3$ [1 : 0.05 : 0.05 v/v/v]; s, strong ROE; m, medium ROE; w, weak ROE).

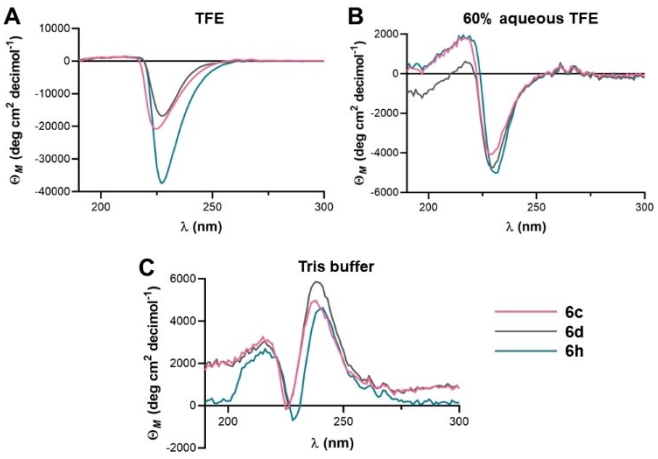


Figure 4. CD spectra of selected oligomers **6c**, **6d** and **6h** in. A) TFE, B) 60% aqueous TFE, and C) aqueous solution (5 mM Tris buffer/150 mM NaCl pH 7.2) at room temperature.

the CD-spectra shown in Figure 4 we observed a conformational change of the peptides in the aqueous buffer-TFE mixture compared to aqueous buffer (Figure 4C), which allows the assumption that the presence of a membrane is necessary for folding and therefore may be important for the antimicrobial/anticancer activity. These findings are in accordance with the literature. For instance, Gellman *et al.*,[23] DeGrado *et al.*[22,24] and Barron *et al.*[25] demonstrated the ability of model membranes to induce a helical conformation of α-peptides as well as ß-peptides.

Antimicrobial and Anticancer Activity

Antibacterial effects of hybrid peptides composed of alternating L-α-amino acids and D-α-aminoxy acids are likely because of the helical conformation adopted by these peptides, which may allow their integration into bacterial membranes and eventually pore formation as bactericidal effect.[2,16] According to the NMR (Figure 3) and CD data (Figure 4), our hybrid peptides are capable of folding into defined secondary structure. Other groups investigated the relationship between helical propensity and antimicrobial activity of membrane-active peptides with different results depending on the peptide type. So far, no clear conclusion can be drawn regarding the relationship of helical conformation and antimicrobial activity.[23,26–28] We thus decided to investigate the antimicrobial properties of this series of hybrid peptides.

Minimal inhibitory concentration (MIC) was determined in cation-adjusted Mueller-Hinton broth (MHB2) following CLSI guidelines[29] to reveal antimicrobial activity of the oligopeptides against the Gram-negative bacteria families Enterobacteriaceae *E. coli, K. pneumoniae*) and Pseudomonadales (*A. baumannii, P. aeruginosa*) as well as against the Gram-positive bacterium *S. aureus* (Table 2). These germs represent priority pathogens according to the World Health Organisation.[30] The studied hybrid peptides differed in peptide chain length (n = 1–8 dimer units) due to iterative addition of the L-Lys-D-^{NO}Phe dimer. If pore formation is the main mode of action, a minimal peptide length is necessary to form helices long enough to cross bacterial membranes. Therefore, one can assume that antimicrobial activity increases with peptide length and elongation of the peptide chain beyond a certain amino acid count should not provide any more benefits to antimicrobial activity. Compounds **6a**–**6d** containing one to four dimers were inactive against all tested bacteria. First antimicrobial activity was detected for decamer **6g** against *E. coli* (64 mg/L) and *S. aureus* (128 mg/L). Dodecamer **6h** showed fourfold lower MIC values for *E. coli* (16 mg/L) and *S. aureus* (32 mg/L) and is active against *A. baumannii* (64 mg/L) and *P. aeruginosa* (128 mg/L). Antimicrobial activity against *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* further increased with total chain lengths of 14 and 16 residues (**6i** and **6j**), confirming our assumption that antimicrobial activity increases with longer chain length.

The effect of different *N*-terminal caps (acetyl- and Fmoc-groups) versus a free *N*-terminus was evaluated for the decamers. Within these peptides, Fmoc-capped **6e** emerged as the most promising compound with high activity against all tested bacteria – *E. coli* (8 mg/L), *P. aeruginosa* (16 mg/L), *K. pneumoniae* (16 mg/L), *A. baumannii* (16 mg/L), and *S. aureus* (4 mg/L). In contrast, decamer **6f** with a free *N*-terminus and the acetylated analogue **6g** showed very weak antimicrobial activity against *E. coli* (64 mg/L) and *S. aureus* (128 mg/L), while being inactive against other tested bacteria. Presumably, the N-terminal Fmoc protection group mainly conveys the antimicrobial activity of **6e**. These findings go in line with studies showing antimicrobial effects of Fmoc-conjugated amino acids against *S. aureus* by alteration of membrane integrity as a lethal event.[31] Furthermore, this supports the observation of DeGrado *et al.* that the hydrophobic Fmoc group favored the binding to dodecyl phosphocholine (DPC) micelles and the corresponding peptides bearing the Fmoc group showed increased antimicrobial activity.[23]

Decamer **7** consists entirely of natural L-amino acids and shows higher antimicrobial activity against all tested bacteria compared to its hybrid peptide counterpart **6g**. Nevertheless, unfavorable conformational flexibility and inferior enzymatic stability (Figure 2B) make **7** a non-ideal therapeutic agent despite its encouraging antimicrobial activity.

Table 2. Minimal inhibitory concentration [mg/L] of hybrid peptides **6a**–**j** and peptide **7** determined in 25% MHB2 and inhibition of proliferation in the esophageal adenocarcinoma cell line OE33.

Peptide	Sequence	Antimicrobial Activity MIC [mg/L]					Anticancer Activity[a] OE33 IC_{50}	
		P. aeruginosa	*K. pneumoniae*	*E. coli*	*A. baumannii*	*S. aureus*	[mg/L]	[μM]
6a	Ac-NH-L-Lys-D-^{NO}Phe-$CONH_2$	>128	>128	>128	>128	>128	n.d.	n.d.
6b	Ac-NH-(L-Lys-D-^{NO}Phe)$_2$-$CONH_2$	>128	>128	>128	>128	>128	n.d.	n.d.
6c	Ac-NH-(L-Lys-D-^{NO}Phe)$_3$-$CONH_2$	>128	>128	>128	>128	>128	n.d.	n.d.
6d	Ac-NH-(L-Lys-D-^{NO}Phe)$_4$-$CONH_2$	>128	>128	>128	>128	>128	n.d.	n.d.
6e	**Fmoc-NH-(L-Lys-D-^{NO}Phe)$_5$-$CONH_2$**	**16**	**16**	**8**	**16**	**4**	**27.2±3.4**	**12±1.5**
6f	H_2N-(L-Lys-D-^{NO}Phe)$_5$-$CONH_2$	>128	>128	64	>128	128	>432	>200
6g	Ac-NH-(L-Lys-D-^{NO}Phe)$_5$-$CONH_2$	>128	>128	64	>128	128	>417	>200
6h	Ac-NH-(L-Lys-D-^{NO}Phe)$_6$-$CONH_2$	128	>128	16	64	32	252±18.4	101±7.4
6i	Ac-NH-(L-Lys-D-^{NO}Phe)$_7$-$CONH_2$	64	128	16	32	16	223±37.7	77±13
6j	**Ac-NH-(L-Lys-D-^{NO}Phe)$_8$-$CONH_2$**	**32**	**32**	**16**	**16**	**16**	**168±39.6**	**51±12**
7	Ac-NH-(L-Lys-L-Phe)$_5$-$CONH_2$	32	32	32	32	16	150±50.1	75±25

[a] n.d.: not determined.

To investigate the anticancer activity all synthesized peptides (**6a–j** and **7**) were tested in proliferation assays in the esophageal adenocarcinoma cell line OE33 at a fixed concentration of 100 μM in a primary screen. In good agreement with data from the antimicrobial activity assays, only peptides with longer chain lengths were able to inhibit the proliferation in OE33 cells. Compounds with anti-proliferative activity in the primary screen (**6e–j** and **7**) were subsequently tested in dose-response assays to determine IC_{50} values. The results are summarized in Table 2.

All compounds were found to have only moderate to poor anti-proliferative activity. However, the highest activities were observed for the Fmoc-capped peptide **6e** (OE33 IC_{50}: 12 μM), followed by **6j** (OE33 IC_{50}: 51 μM), which is the hybrid peptide with the longest chain length in this series. These results indicate that a further elongation of the chain length and modification of the *N*-terminal cap might be important to increase the anti-carcinogenic potential of our hybrid peptides in the future.

Conclusion

In conclusion, we have synthesized a mini library of hybrid peptides containing alternating amino and α-aminoxy acids in order to study their conformational properties and their potential as antimicrobial and anticancer foldamers. To investigate the benefits of this backbone regarding the stability towards enzymatic degradation, we synthesized a decamer containing only α-amino acids. The comparison of the stability against trypsin clearly confirmed the enhancement of the enzymatic stability through the incorporation of the α-aminoxy acids. Conformational studies by NMR spectroscopy were in good agreement with previously published data, showing a characteristic ROE pattern, which indicates the presence of iterative α-N–O-turns and γ-turns leading to a 7/8 helical conformation. Furthermore, a significant influence of peptide chain length on antimicrobial activity could be confirmed. A minimum chain length of ten amino acids was required to inhibit bacterial growth. Modifications of the *N*-terminal position demonstrated that the Fmoc-capped hybrid peptide **6e** displayed significant higher antimicrobial and anti-carcinogenic activity compared to cap-less and acetylated analogues. Taken together, this series of hybrid peptides based on α-aminoxy acids and α-amino acids represents a promising starting point for the development of proteolytically stable foldamers with improved antimicrobial and anticancer activities.

Experimental Section

General Information

Materials and Methods: All chemicals were obtained from commercial suppliers and used as purchased without further purification. Solvents with technical grade were distilled prior to use. Acetonitrile in HPLC-grade quality (HiPerSolv CHROMANORM, *VWR*) was used for all HPLC purposes. Water was purified with a Milli-Q Simplicity 185 Water Purification System (Merck Millipore). Thin-layer chromatography (TLC) was carried out on prefabricated plates (silica gel 60, F254 with fluorescence indicator, *Merck*). Components were visualized by irradiation with ultraviolet light (254 nm, 366 nm) or by staining in potassium permanganate dip or ninhydrin dip followed by careful heating (~300 °C). Column chromatography was either carried out on silica gel (NORMASIL 60®, 40–63 μm, *VWR*) or on a *Teledyne ISCO* Combi Flash NEXTGEN 300+ using prepacked silica columns (Redisep® normal phase, 35 to 70 microns, 230 to 400 mesh, 60 Å in the sizes 12 g or 24 g, *Teledyne ISCO*). Figure 1 was created with BioRender.com.

Nuclear Magnetic Resonance Spectroscopy (NMR): Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded either on *Bruker* Avance III HD 400 MHz at a frequency of 400 MHz (^{1}H) and 100 MHz (^{13}C) or *Varian/Agilent* Mercury-plus-400 at a frequency of 400 MHz (^{1}H) and 100 MHz (^{13}C) or a *Varian/Agilent* Mecury-plus-300 at a frequency of 300 MHz (^{1}H) and 75 MHz (^{13}C). The 2D NMR spectra have been acquired on a *Bruker* Avance NEO instrument operating at 600 MHz (^{1}H frequency). The residual solvent signal ($CDCl_3$: ^{1}H-NMR: 7.26 ppm, ^{13}C-NMR: 77.1 ppm, DMSO-d_6: ^{1}H-NMR: 2.50 ppm, ^{13}C-NMR: 39.5 ppm, D_3COD: ^{1}H-NMR: 3.30, 4.79 ppm ^{13}C-NMR: 49.0 ppm) was used for calibration referred to tetramethylsilane. The chemical shifts are given in parts per million (ppm). The multiplicity of each signal is reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) or combinations thereof. Multiplicities are reported as they were measured, and might disagree with the expected multiplicity of a signal.

Mass Spectrometry: High-resolution electrospray ionisation mass spectra (HR-ESI-MS) were acquired either with a Bruker Daltonik GmbH micrOTOF coupled to a LC Packings Ultimate HPLC system and controlled by micrOTOFControl3.4 and HyStar 3.2-LC/MS or with a Bruker Daltonik GmbH ESI-qTOF Impact II coupled to a Dionex UltiMate™ 3000 UHPLC system and controlled by micrOTOFControl 4.0 and HyStar 3.2-LC/MS.

CD spectroscopy: The JASCO J-1500 instrument was used to record the CD spectra. The spectrometer was equilibrated at 25 °C for 30 min and the measuring chamber was flushed with nitrogen gas. Prior to each measurement, the blank signal of the solvent was acquired. For measurements in TFE, each peptide was dissolved in TFE (**6c**: 228 μM, **6d**: 238 μM, **6h**: 233 μM) and measured using a quartz cuvette with 1 cm path length. For measurements in aqueous solution, 100 μL freshly prepared 2 mM stock solutions of the peptides in millipore water were diluted with 900 μL Tris buffer or 60% TFE in Tris buffer to yield a 200 μM peptide solution.

High Performance Liquid Chromatography (HPLC): For analytical purposes either a Thermo Fisher Scientific UltiMate™ 3000 UHPLC system or a Gynkotek Gina 50 HPLC system (Detector: Gynkotek UVD340 U, Pump: Dionex P680 HPLC pump, column oven: Dionex STH 585) with a Nucleodur 5 μm C18 100 Å (250×4.6 mm, Macherey Nagel) column were used. In the process a flow rate of 1 mL/min and a temperature of 25° C were set. For preparative purposes a Varian ProStar system with either a Jupiter 5 μm C18 100 Å-column (250×10 mm, Phenomenex) with 4 mL/min or a Nucleodur 5 μm C18 HTec (150×32 mm, Macherey Nagel) column with 15/20 mL/min were used. Detection was implemented by UV absorption measurement at a wavelength of λ=220 nm. purified H_2O (A) and MeCN (B) were used as eluents with an addition of 0.1% TFA for eluent A. For analytical as well as preparative purposes after column equilibration for 5 min a linear gradient from 5% A to 95% B in 30 min was used.

Synthesis

Fmoc-NH-L-Lys(Boc)-D-^{NO}Phe-OtBu (**3**): Hydrazine monohydrate (0.34 mL, 6.90 mmol, 3.00 eq) was added to a solution of Phth-D-^{NO}Phe-O*t*Bu (843 mg, 2.30 mmol, 1.10 eq) in MeOH (11.5 mL). The reaction was stirred for 1.5 h at room temperature. Upon full conversion, the solvent was subsequently removed under reduced pressure. The crude product was dissolved in 5% aq. Na_2CO_3 solution (10 mL) and extracted with Et_2O (3×15 mL). The combined organic phases were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford the phthaloyl deprotected crude monomer as colorless oil, which was used without further purification. The crude product was dissolved in dry CH_2Cl_2 (5 mL) and added gently dropwise to a stirred solution of Fmoc-NH-L-Lys(Boc)-OH (1.00 g, 2.10 mmol, 1.00 eq), $HOBt \cdot H_2O$ (386 mg, 2.52 mmol, 1.20 eq) and EDC·HCl (483 mg, 2.52 mmol, 1.20 eq) in dry CH_2Cl_2 (20 mL). The reaction was stirred for 18 h at room temperature. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (cyclohexane/EtOAc) using a linear gradient from 5–95% EtOAc in 30 min yielding the desired product **3** as colorless solid (1.39 g, 2.02 mmol, 96%). **Mp.**: 66.3–71.8 °C. **^{1}H-NMR** (400 MHz, DMSO-d_6, δ): 11.30 (s, 1H), 7.88 (dd, J=7.6, 1.1 Hz, 2H), 7.71 (dd, J=7.5, 4.5 Hz, 2H), 7.55 (d, J=8.1 Hz, 1H), 7.41 (tt, J=7.5, 1.4 Hz, 2H), 7.32 (td, J=7.4, 1.2 Hz, 2H), 7.25 (qd, J=8.5, 7.8, 4.1 Hz, 5H), 6.75 (t, J=5.9 Hz, 1H), 4.50 (t, J=6.6 Hz, 1H), 4.26–4.17 (m, 3H), 3.83 (q, J=7.5 Hz, 1H), 3.10–2.94 (m, 2H), 2.95–2.81 (m, 2H), 1.53 (q, J=7.4 Hz, 2H), 1.36 (s, 9H), 1.35–1.28 (m, 2H), 1.26 (s, 9H) 1.24–1.08 (m, 2H).**^{13}C-NMR** (101 MHz, DMSO-d_6, δ): 169.4, 168.9, 155.8, 155.6, 143.9, 143.8, 140.7, 129.4, 128.1, 127.6, 127.1, 126.6, 125.3, 120.1, 83.1, 81.1, 77.3, 65.6, 52.2, 46.6, 36.5, 31.3, 29.1, 28.3, 27.5, 27.5, 22.7 ppm. **HR-ESI-MS (*m/z*)**: $[M+Na]^+$ calcd for $C_{39}H_{49}N_3O_8$: 710.3412, found: 710.3422. **IR**: $\tilde{\nu}$=3319 (br), 2976 (br), 2932 (br), 1673 (s), 1513 (br, m), 1450 (m), 1392 (w), 1366 (m), 1246 (s), 1154 (s), 1041 (m, br), 843 (w), 758 (m), 739 (s), 699 (m), 621 (w), 544 (w), 462 (w) cm^{-1}.

Fmoc-NH-L-Lys(Boc)-D-^{NO}Phe-OH (4): TFA (10 mL) was added dropwise over a period of 15 min to a cooled (0 °C) solution of **3** (1.39 g, 2.02 mmol, 1.00 eq) in CH_2Cl_2 (30 mL). The reaction was then allowed to warm to room temperature and stirred for further 2 h. The solvent was removed under reduced pressure to afford the deprotected dimer as yellowish oil, which was used without further purification. To a solution of the crude product in CH_2Cl_2 (40 mL) was added DIPEA (1.40 mL, 8.08 mmol, 4.00 eq) and Boc_2O (561 µL, 2.62 mmol, 1.30 eq). After stirring for 6 h the reaction was quenched gently with 10% aq. citric acid. The organic phase was washed consecutively with 10% aq. citric acid (3×40 mL) and with brine (3×40 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH) using a linear gradient from 0–10% MeOH in 30 min yielding the desired product **4** as colourless solid (1.04 g, 1.65 mmol, 82%). **Mp.**: 102.8–109.2 °C. **^{1}H-NMR**: (300 MHz, DMSO-d_6, δ): 7.94–7.84 (m, 2H), 7.72 (dd, J=7.5, 3.7 Hz, 2H), 7.55 (d, J=8.0 Hz, 1H), 7.47–7.36 (m, 2H), 7.35–7.15 (m, 7H), 6.85–6.67 (m, 1H), 4.50 (t, J=6.0 Hz, 1H), 4.22 (q, J=4.6, 4.2 Hz, 3H), 3.85 (d, J=7.6 Hz, 1H), 3.03 (dd, J=6.1, 3.4 Hz, 2H), 2.88 (d, J=6.5 Hz, 2H), 1.49 (dd, J=19.7, 6.6 Hz, 2H), 1.36 (s, 9H), 1.32–1.12 (m, 4H) ppm. **^{13}C-NMR** (101 MHz, DMSO-d_6, δ): 155.8, 155.6, 143.9, 143.8, 140.7, 139.4, 137.4, 129.3, 128.9, 127.9, 127.6, 127.1, 125.4, 120.1, 83.8, 77.3, 65.7, 52.6, 46.6, 45.3, 36.8, 29.1, 28.3, 22.8, 9.3 ppm. **HR-ESI-MS (*m/z*)**: $[M+Na]^+$ calcd for $C_{35}H_{41}N_3O_8$: 654.2786, found: 654.2793. **IR**: $\tilde{\nu}$=3301 (br), 2934 (br), 1681 (s), 1513 (br, m), 1450 (m), 1365 (w), 1247 (s), 1165 (s), 1032 (br, m), 861 (w), 758 (m), 739 (s), 698 (m), 620 (w), 542 (w) cm^{-1}.

Solid Phase Synthesis

General Information: For synthesis carried out on solid phase either Fmoc-Sieber-PS resin (100–200 mesh, 0.59 mmol/g) or Fmoc-Rink-Amide PEG AM resin (200–400 mesh, 0.56 mmol/g) supplied by Iris Biotech was used. All non-modified amino acids were used as commercially available Fmoc-L-amino acids supplied by Carbolution Chemicals GmbH. The manual peptide synthesis was carried out in PP-reactors with PE frit (sizes: 2/10/20 mL, pore size 25 µm, Multi-SynTech GmbH). All syntheses were following the standard Fmoc-solid phase method. After resin swelling for 30 min in DMF the standard procedure was carried out by repeating the following steps: – Fmoc-deprotection – coupling of the amino acid – capping of the left free *N*-termini. Completion of each coupling step was monitored via TNBS-test using a TNBS test kit supplied by TCI. In the case of the hybrid peptides the dimeric building block **4** synthesized before in solution was used in solid phase synthesis following the standard Fmoc-solid phase method. In the case of peptide **7** the commercially available Fmoc-L-amino acids were used as monomeric building blocks. After the last coupling cycle was carried out the steps Fmoc-deprotection and capping were conducted to give the desired peptides with acetylated termini, except in the cases of **6e** and **6f**, where the synthesis was stopped after the last coupling cycle (for the Fmoc-cap) and after the Fmoc deprotection (for free *N*-terminus). For purification the peptides were cleaved from the resin using a suitable cleavage cocktail and the final peptide was purified by preparative RP-HPLC. Fractions containing the desired peptides were collected and lyophilized yielding the peptides with >95% purity in all cases. The amounts of reagents and solvents used in the following synthesis protocol correspond to 10–80 µmol scale.

Fmoc-deprotection: Piperidine (20% in DMF, 500 µL) was added to the resin and the syringe was shaken for 5 min. The step was repeated once followed by a washing step with DMF (5×1.5 mL), CH_2Cl_2 (5×1.5 mL) and DMF (5×1.5 mL). After the washing step the needle was changed to prevent reactions with extant piperidine.

Coupling: Fmoc-protected dimeric building block **4** (3.00 eq) was dissolved in a solution of Oxyma in DMF (0.5 M, 3.00 eq). HATU (3.00 eq) and DIPEA (4.00 eq) were added and the solution was activated through shaking for 3 min and afterwards added to the resin. Amide coupling was performed for 90 min at room temperature. After washing with DMF (5×1.5 mL) and CH_2Cl_2 (5×1.5 mL), the TNBS-test was implemented and the resin was washed with DMF (5×1.5 mL). In the case of a positive TNBS-test the coupling step was repeated after washing with DMF (5×1.5 mL).

TNBS-test: A little amount of resin-beads was placed in a 0.5 mL micro centrifuge tube. One drop of picrylsulfonic acid (~1% in DMF) and two drops of DIPEA (10% in DMF) were added to the resin beads and the resin beads were reacted for 3 min at room temperature.

Capping: 1 mL of the capping solution (Lutidine/Ac_2O/DMF, 6:5:89, *v/v/v*) was added to the resin and the mixture was agitated for 5 min at room temperature. After filtration the resin was washed with DMF (5×1.5 mL), CH_2Cl_2 (5×1.5 mL) and DMF (5×1.5 mL).

Test cleavage/Cleavage from the resin: For test cleavage about 2 mg of the resin was weighed into a tube and treated with the desired cleavage solution for one or two hours. After the reaction period was finished, the peptides were precipitated in cold Et_2O for 30 min and centrifuged afterwards (5 min, 13.000 rpm). The pellet was solvated in milliQ H_2O/MeCN for analytical purposes via HPLC. A cleavage on a larger scale was carried out in the same way. For each 40 mg of resin 1.0 mL of cleavage cocktail was used. For synthesis conducted on rink amide PEG AM resin cleavage solution A (TFA/CH_2Cl_2/TIPS, 94:5:1, v/v/v) was used, whereas peptides

synthesized on sieber amide PS resin were cleaved with cleavage solution B (TFA/CH_2Cl_2/TIPS, 5:94:1, v/v/v).

Ac-NH-L-Lys-D-^{NO}Phe-CONH$_2$ (6a): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 50.0 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid dimer. Purification by preparative RP-HPLC gave the desired product (3.70 mg, 7.97 μmol, 16%) as colourless solid. **HPLC**: t_R: 14.35 min, 98.6%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{17}H_{26}N_4O_4$: 351.2027, found: 351.2031.

Ac-NH-(L-Lys-D-^{NO}Phe)$_2$-CONH$_2$ (6b): Sieber amide PS resin with a loading of 0.59 mmol/g was used. The synthesis was carried out in 80.0 μmol scale using the general procedures described before. Cleavage cocktail B was used for the cleavage of the hybrid tetramer. Purification by preparative RP-HPLC gave the desired product (14.0 mg, 16.1 μmol, 20%) as colourless solid. **HPLC**: t_R: 16.34 min, 97.5%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{32}H_{47}N_7O_7$: 642.3610, found: 642.3616.

Ac-NH-(L-Lys-D-^{NO}Phe)$_3$-CONH$_2$ (6c): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 44.0 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid hexamer. Purification by preparative RP-HPLC gave the desired product (30.1 mg, 23.6 μmol, 54%) as colourless solid. **HPLC**: t_R: 17.45 min, >99.5%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{47}H_{68}N_{10}O_{10}$: 933.5193, found: 933.5185.

Ac-NH-(L-Lys-D-^{NO}Phe)$_4$-CONH$_2$ (6d): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 44.0 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid octamer. Purification by preparative RP-HPLC gave the desired product (38.0 mg, 22.6 μmol, 51%) as colourless solid. **HPLC**: t_R: 17.71 min, 99.2%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{62}H_{89}N_{13}O_{13}$: 1224.6776, found: 1224.6780.

Fmoc-NH-(L-Lys-D-^{NO}Phe)$_5$-CONH$_2$ (6e): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 65.0 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid peptide. Purification by preparative RP-HPLC gave the desired product (6.30 mg, 2.78 μmol, 4%) as colourless solid. **HPLC**: t_R: 19.82 min, 96.0%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{90}H_{118}N_{16}O_{17}$: 1696.8967, found: 1696.8956.

H$_2$N-(L-Lys-D-^{NO}Phe)$_5$-CONH$_2$ (6f): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 12.5 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid peptide. Purification by preparative RP-HPLC gave the desired product (3.22 mg, 1.49 μmol, 12%) as colourless solid. **HPLC**: t_R: 16.86 min, 95.2%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{75}H_{108}N_{16}O_{15}$: 1473.8253, found: 1473.8254.

Ac-NH-(L-Lys-D-^{NO}Phe)$_5$-CONH$_2$ (6g): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 12.5 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid peptide. Purification by preparative RP-HPLC gave the desired product (7.10 mg, 3.40 μmol, 27%) as colourless solid. **HPLC**: t_R: 17.24 min, 98.9%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{77}H_{110}N_{16}O_{16}$: 1515.8358, found: 1515.8362.

Ac-NH-(L-Lys-D-^{NO}Phe)$_6$-CONH$_2$ (6h): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 44.0 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid peptide. Purification by preparative RP-HPLC gave the desired product (50.0 mg, 20.1 μmol, 46%) as colourless solid. **HPLC**: t_R: 17.79 min, 99.1%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{92}H_{131}N_{19}O_{19}$: 1806.9941, found: 1806.9951.

Ac-NH-(L-Lys-D-^{NO}Phe)$_7$-CONH$_2$ (6i): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 25.0 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid peptide. Purification by preparative RP-HPLC gave the desired product (5.20 mg, 1.80 μmol, 7%) as colourless solid. **HPLC**: t_R: 17.10 min, 95.3%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{107}H_{152}N_{22}O_{22}$: 2099.1558, found: 2099.1558.

Ac-NH-(L-Lys-D-^{NO}Phe)$_8$-CONH$_2$ (6j): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 25.0 μmol scale using the general procedures described before. Cleavage cocktail A was used for the cleavage of the hybrid peptide. Purification by preparative RP-HPLC gave the desired product (6.10 mg, 1.85 μmol, 7%) as colourless solid. **HPLC**: t_R: 16.98 min, 97.4%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{122}H_{173}N_{25}O_{25}$: 2390.3141, found: 2390.3146.

Ac-NH-(L-Lys-L-Phe)$_5$-CONH$_2$ (7): Rink amide PEG AM resin with a loading of 0.56 mmol/g was used. The synthesis was carried out in 50.0 μmol scale using the general procedures described before. In this case for amino acid coupling Fmoc-L-aa-OH (4.00 eq) was dissolved in a solution of oxyma in DMF (0.5 M, 4.00 eq) and DIC (4.00 eq) was added. The resulting mixture was activated through shaking for 3 min. The activated solution was added to the resin and the coupling was performed for 60 min at room temperature. Cleavage cocktail A was used for the cleavage of the α-peptide decamer. Purification by preparative RP-HPLC gave the desired product (36.1 mg, 18.0 μmol, 36%) as colourless solid. **HPLC**: t_R: 16.97, >99.5%. **HR-ESI-MS (*m/z*)**: $[M+H]^+$ calcd for $C_{77}H_{110}N_{16}O_{11}$: 1435.8613, found: 1435.8608.

Solubility assay

A certain amount (~1 mg) of the peptides was suspended/dissolved in different solvents to yield a 10 mM solution or suspension, which was shaken 30 min at 1000 rpm at 25 °C on a Thermomixer comfort (Eppendorf), centrifuged at 14000 rpm at 25 °C for 60 min with a Heraeus Fresco 21 Centrifuge (Thermo Scientific). The supernatant was diluted 1 to 30 (in case of **6h** 1 to 40) and measured by HPLC, using a linear gradient from 5% A to 95% B in 7 min after column equilibration for 3.5 min with a flow rate of 1.5 mL/min. Concentrations were determined in duplicate series.

Calibration curve: a 10 mM stock solution of each peptide in 20% ACN in water was diluted to 500 μM, 400 μM, 300 μM, 200 μM, 100 μM, 50 μM, 25 μM and 10 μM using the same solvent mixture. For each peptide two weighed portions were used for each stock solution. 25 μL of diluted solutions were injected and each sample was measured twice. For quantitative analysis, only peak areas of data points with a peak height <1000 mAU were taken into account for the linear regression.

Stability assay

All experiments have been carried out in triplicate series. An assay end volume of 25 μL was set consisting of 20 μL assay buffer (10 mM Tris buffer, pH 7.5), 4.15 μL of the peptide in assay buffer (2 mM) and 0.85 μL trypsin (0.02 mg/mL in assay buffer). At specific time points 50 μL of the stop solution (5% TFA in millipore H_2O (v/v)) was added to the reaction. In case of the chemical stability

assay trypsin solution was replaced by assay buffer and the reaction was not quenched. Stabilities at each time point were analyzed by RP-HPLC, using a linear gradient from 5% A to 95% B in 7 min after column equilibration for 3.5 min with a flow rate of 1.5 mL/min.

Antimicrobial Activity

Materials: Mueller-Hinton broth 2 (MHB2, for microbiology, cation-adjusted, Sigma-Aldrich). Water was purified on a Purelab Ultra water purification system (electrical resistivity $>182\ k\Omega\cdot m$; organic content <2 ppb; ELGA LabWater, Celle, Germany). The following bacteria were used: *Escherichia coli* DSM 1103/ATCC® 25922™, *Pseudomonas aeruginosa* DSM 1117/ATCC® 27853™, *Klebsiella pneumoniae* DSM 681/ATCC® 10031™, *Acinetobacter baumannii* DSM 30008/ATCC® 15308™, *Staphylococcus aureus* DSM 6247 (DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany).

Method: Minimal inhibitory concentration (MIC) was determined in 25% cation-adjusted Mueller-Hinton broth (25% MHB2; 5.5 g/L) using a broth microdilution assay. Experiments were carried out in triplicate in 96 well plates (polystyrene F-bottom; ref. 655180, Greiner Bio-One GmbH, Frickenhausen, Germany) and repeated at least once on another day. Aqueous peptide solutions (3 g/L) were serially twofold diluted in eight steps starting at a concentration of 128 mg/L. Bacteria were grown overnight (16–18 hours, 200 rpm, 37 °C) in 25% MHB2 followed by 30-fold dilution in fresh broth. After an incubation period of four hours (200 rpm, 37 °C), cell count was adjusted according to McFarland standard to obtain a final concentration of 7.5×10^6 cells/mL. The plates were incubated at 37 °C for 20 ± 2 hours. Bacterial growth was detected by measuring optical density at 595 nm using a microplate reader (Victor3™, PerkinElmer Inc., Waltham, MA, USA). MIC was defined as the lowest peptide concentration inhibiting visible bacterial growth.

Cell culture

OE33 cells were purchased from Sigma Aldrich (ECACC: 96070808, Taufkirchen, Germany) and were cultured as described previously.[32]

OE33 (esophageal adenocarcinoma – EAC) cells were seeded in 96 well plates (polystyrene F-bottom; ref. 655180, Greiner Bio-One GmbH, Frickenhausen, Germany) and were treated with compounds **6a–j** and **7** for 48 h. Cell viability was determined by PrestoBlue Cell Viability Reagent (ThermoFisher, Darmstadt, Germany) accordingly to the manufacturer's protocol.

Acknowledgements

The authors would like to thank Franziska Rolfs and Katrin Steinke for their excellent technical support. We thank the Institute of Biochemistry at Leipzig University for providing us access to the CD spectrometer. Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

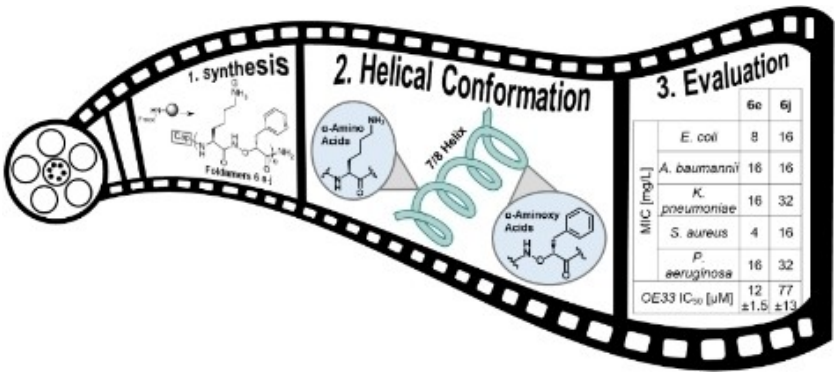
The authors declare no conflict of interest.

Keywords: α-aminoxy acids · antimicrobial activity · anticancer peptides · foldamers · peptidomimetics

[1] C. M. Crews, *Chem. Biol.* **2010**, *17*, 551–555.
[2] X. Li, Y. D. Wu, D. Yang, *Acc. Chem. Res.* **2008**, *41*, 1428–1438.
[3] K. Fosgerau, T. Hoffmann, *Drug Discovery Today* **2015**, *20*, 122–128.
[4] S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180.
[5] M. F. Maitz, *Biosurface and Biotribology* **2015**, *1*, 161–176.
[6] D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893–4011.
[7] D. Diedrich, A. J. R. Moita, A. Rüther, B. Frieg, G. J. Reiss, A. Hoeppner, T. Kurz, H. Gohlke, S. Lüdeke, M. U. Kassack, F. K. Hansen, *Chem. A Eur. J.* **2016**, *22*, 17600–17611.
[8] D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, *1*, 2015–2022.
[9] D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 13071–13072.
[10] D. Yang, F. F. Ng, Z. J. Li, Y. D. Wu, K. W. K. Chan, D. P. Wang, *J. Am. Chem. Soc.* **1996**, *118*, 9794–9795.
[11] F. Chen, N. Y. Zhu, D. Yang, *J. Am. Chem. Soc.* **2004**, *126*, 15980–15981.
[12] F. Chen, B. Ma, Z. C. Yang, G. Lin, D. Yang, *Amino Acids* **2012**, *43*, 499–503.
[13] D. Yang, G. J. Liu, Y. Hao, W. Li, Z. M. Dong, D. W. Zhang, N. Y. Zhu, *Chem. Asian J.* **2010**, *5*, 1356–1363.
[14] D. Yang, J. Qu, B. Li, F. F. Ng, X. C. Wang, K. K. Cheung, D. P. Wang, Y. D. Wu, *J. Am. Chem. Soc.* **1999**, *121*, 589–590.
[15] S. Bhatia, D. Diedrich, B. Frieg, H. Ahlert, S. Stein, B. Bopp, F. Lang, T. Zang, T. Kröger, T. Ernst, G. Kögler, A. Krieg, S. Lüdeke, H. Kunkel, A. J. Rodrigues Moita, M. U. Kassack, V. Marquardt, F. V. Opitz, M. Oldenburg, M. Remke, F. Babor, M. Grez, A. Hochhaus, A. Borkhardt, G. Groth, L. Nagel-Steger, J. Jose, T. Kurz, H. Gohlke, F. K. Hansen, J. Hauer, *Blood* **2018**, *132*, 307–320.
[16] X. Li, D. Yang, *Chem. Commun.* **2006**, 3367–3379.
[17] M. Mahlapuu, J. Håkansson, L. Ringstad, C. Björn, *Front. Cell. Infect. Microbiol.* **2016**, *6*, 1–12.
[18] D. Gaspar, A. Salomé Veiga, M. A. R. B. Castanho, *Front. Microbiol.* **2013**, *4*, 1–16.
[19] M. R. Felício, O. N. Silva, S. Gonçalves, N. C. Santos, O. L. Franco, *Front. Chem.* **2017**, *5*, 1–9.
[20] D. Yang, W. Li, J. Qu, S. W. Luo, Y. D. Wu, *J. Am. Chem. Soc.* **2003**, *125*, 13018–13019.
[21] A. Hollmann, M. Martinez, P. Maturana, L. C. Semorile, P. C. Maffia, *Front. Chem.* **2018**, *6*, 1–13.
[22] T. L. Raguse, E. A. Porter, B. Weisblum, S. H. Gellman, *J. Am. Chem. Soc.* **2002**, *124*, 12774–12785.
[23] W. F. DeGrado, J. D. Lear, *J. Am. Chem. Soc.* **1985**, *107*, 7684–7689.
[24] Y. Hamuro, J. P. Schneider, W. F. DeGrado, *J. Am. Chem. Soc.* **1999**, *121*, 12200–12201.
[25] J. A. Patch, A. E. Barron, *J. Am. Chem. Soc.* **2003**, *125*, 12092–12093.
[26] H. C. Chen, J. H. Brown, J. L. Morell, C. M. Huang, *FEBS Lett.* **1988**, *236*, 462–466.
[27] S. M. Travis, N. N. Anderson, W. R. Forsyth, C. Espiritu, B. D. Conway, E. P. Greenberg, P. B. McCray, R. I. Lehrer, M. J. Welsh, B. F. Tack, *Infect. Immun.* **2000**, *68*, 2748–2755.
[28] A. Tossi, L. Sandri, A. Giangaspero, *Biopolymers* **2000**, *55*, 4–30.
[29] CLSI Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: M07-A8; CLSI: Wayne, PA, USA, 2009.
[30] "World Health Organization. (2017, February 27) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics" can be found under https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/, retrieved 01.12.2020.
[31] A. Y. Gahane, P. Ranjan, V. Singh, R. K. Sharma, N. Sinha, M. Sharma, R. Chaudhry, A. K. Thakur, *Soft Matter* **2018**, *14*, 2234–2244.
[32] O. Chemnitzer, K. Götzel, L. Maurer, A. Dietrich, U. Eichfeld, O. Lyros, B. Jansen-Winkeln, A. Hoffmeister, I. Gockel, R. Thieme, *Dig. Dis. Sci.* **2017**, *62*, 3391–3401.

Manuscript received: December 22, 2020
Revised manuscript received: February 18, 2021
Accepted manuscript online: February 19, 2021

FULL PAPERS



*L. Sinatra, L. Kolano, Dr. M. Icker, S. R. Fritzsche, Dr. D. Volke, Prof. Dr. I. Gockel, Dr. R. Thieme, Prof. R. Hoffmann, Prof. Dr. F. K. Hansen**

Peptidomimetic foldamers consisting of alternating α-aminoxy acids and α-amino acids were synthesized using a combination of solution- and solid-phase supported protocols. 2D ROESY experiments confirmed the presence of a helical conformation in solution. The biological evaluation revealed a significant impact of the peptide chain length and the *N*-terminal cap and demonstrated the utility of these peptides as antimicrobial and anticancer foldamers.