The Role of Terminal Functionality in the Membrane and Antibacterial Activity of Peptaibol-Mimetic Aib Foldamers


Abstract: Peptaibols are peptide antibiotics that typically feature an N-terminal acetyl cap, a C-terminal aminooalcohol, and a high proportion of α-aminoisobutyric acid (Aib) residues. To establish how each feature might affect the membrane-activity of peptaibols, biomimetic Aib foldamers with different lengths and terminal groups were synthesised. Vesicle assays showed that long foldamers (eleven Aib residues) with hydrophobic termini had the highest ionophoric activity. C-terminal acids or primary amides inhibited activity, while replacement of an N-terminal acetyl with an azide group made little difference. Crystallography showed that N₄Aib,CH₃OTIPS folded into a 3₁0 helix 2.91 nm long, which is close to the bilayer hydrophobic width. Planar bilayer conductance assays showed discrete ion channels only for N-acetylated foldamers. However long foldamers with hydrophobic termini had the highest antibacterial activity, indicating that ionophoric activity in vesicles was a better indicator of antibacterial activity than the observation of discrete ion channels.

Introduction

Peptaibols are a class of antimicrobial peptide secreted by Trichoderma fungi.[1] Common structural features of peptaibols include acylated N-termini (acetyl in long peptaibols or C8 to C15 acyl chains in lipopeptaibols[2]), a high proportion of the non-coded amino acid α-aminoisobutyric acid (Aib) and usually (although not exclusively)[3,4] a C-terminal aminooalcohol residue. The prevalence of quarternary residues, such as Aib, promotes folding into 3₁₀- or α-helical secondary structures,[5] and the resulting lipophilic helices can be highly membrane active. The most commonly studied peptaibol, 19-residue alamethicin, forms voltage-dependant ion channels in lipid membranes[6] and this channel formation is implicated in its bactericidal activity.[7] Alamethicin has an N-terminal acetyl group and a C-terminal phenylalaninol residue, and is predominantly α-helical with a length of ca. 2.8 nm.[8] The formation of membrane-spanning pores is thought to depend upon the aggregation of 3 to 12 alamethicin helices in the membrane; the tethering of six or seven alamethicin peptides to a central hub leads to remarkably stable ion channels.[9] Although alamethicin shows good activity against fungi and Gram-positive bacteria, its toxicity to mammalian cells impairs medical applications.[10]

The creation of synthetic channel-forming compounds[11,12] can lead to a better understanding of how membrane-active antibiotics like peptaibols form channels and pores. It may also provide new classes of antibiotics that could help overcome the growing problem of antibiotic resistance.[13] Despite the potential applications of synthetic channel-forming compounds, few studies have elucidated structure-activity relationships that link activity in non-biological membranes with activity against cells,[14] although Gokel and co-workers correlated the channel-forming activity of synthetic hydraphile channels in phospholipid bilayers with activity against bacterial and mammalian cells.[15]

Given that an N-terminal acetyl group and a C-terminal alcohol are such common structural features of the antimicrobial peptaibols, it seems reasonable to propose that these modifications are important for the antibiotic function of these compounds. Both of these features have the potential to hydrogen bond to adjacent residues and thereby stabilise folded secondary structures, such as α- and 3₁₀-helices; in peptaibols both inter- and intramolecular hydrogen bonds to the terminal hydroxyl have been observed.[16,17] It has also been suggested that a hydroxyl group stabilises ion channels by hydrogen bonding to the glycerol and phosphate in phospholipid headgroups.[17] Analogous peptaibol derivatives with esters at the C-terminus are reported to still be membrane active,[18] and in some cases to be as effective as the alcohols.[19] Acetylation at the N-terminus is widespread in peptides and proteins and is known to play many roles, including regulating protein stability, membrane targeting and gene silencing.[20]

Developing synthetic molecular devices able to communicate chemicals or information across membranes is an area of keen interest.[21,22] We have shown that Aib-rich foldamers can act as synthetic ion channels[23] and membrane-spanning signal transducers[24] in the latter case using their dynamic conformational properties to transmit photochemical and chemical information deep into phospholipid bilayers. To extend transduction studies into a cellular environment, it is...
necessary to understand which structural features in these Aib foldamers may promote ionophoric behaviour. In addition, the modular nature of synthetic compounds, in which chain length and chain termini can be modulated freely, makes Aib foldamers valuable model systems for understanding peptaibol behaviour. Having recently shown the importance of Aib foldamer length in determining ionophoric activity, we now sought to investigate how the N-terminal acetyl and C-terminal hydroxyl groups found in channel-forming peptaibols affect the ionophoric activity of Aib foldamers. In an era of increased antibiotic resistance, such studies may allow the design of optimised peptaibol antibiotics.

**Results and Discussion**

**Foldamer synthesis**

![Diagram of foldamer synthesis](image)

We have observed membrane activity for Aib foldamers containing as few as 5 residues, but studies on the $N_{3}Alb_{10}C(O)CH_{2}CH_{2}SiMe_{3}$ compound family ($n = 4$ to 13) showed a strong increase in activity when $n \geq 9$ (Figure 1c). Planar bilayer conductance (PBC) studies showed clear channel-like behaviour for $n = 11$ and 13, but not for $n = 7$ and 8. Nonetheless, this earlier systematic study only examined the behaviour of foldamers bearing hydrophobic termini.

To allow comparisons to be made with the peptaibols, we synthesised new foldamers 2 - 4, 6 - 10, 12 - 15 (Figure 1b), with lengths on either side of the apparent nine-residue cut-off for good membrane activity but also containing functional groups commonly found in the naturally occurring peptaibols (acetyl at N-terminus, hydroxyl at C-terminus). As control compounds, we also assessed foldamers 1, 5, 11, 16 – 18, which had non-polar functional groups at both termini that cannot participate in a $3_{10}$ helix hydrogen-bonding network.

![Diagram of X-ray crystal structures](image)

Choosing only three lengths of peptides to assess, namely “Aib”, “Aib$_2$” and “Aib$_3$”, simplified the synthetic routes to these compounds. The synthetic routes started from the tetramer peptide, $N_{3}Alb_{4}OH$ 19, which was then coupled to the protected 1,2-aminoalcohol $H_{2}NCMe_{2}CH_{2}OTIPS$ (“Aib$_{11}$CH$_2$OTIPS”) to give compound 1. This compound was...
either deprotected at the C-terminus to release the free alcohol 3, reduced then acetylated at the N-terminus to give 2, or reduced then coupled with N₂AibOH trimer to give 5. This chain extension gave access to the “Aib₃” and “Aib⁺” series of compounds after analogous chemical transformations. Compounds 1, 5 and 11 have non-polar groups at both termini, and additional non-polar reference compounds 16, 17 and 18 were synthesised according to literature methods.[23]

The solid-state structure of a foldamer with non-polar termini, Aib₃Aib₃CH₂O-OTIPS 11 (Figure 2a), shows that the Aib core folds into a 3₁₀-helical secondary structure with the silylated tail adopting an extended conformation. The foldamer has an end-to-end distance of 2.91 nm, which is commensurate with the hydrophobic width of a phospholipid bilayer (e.g. EYPC:cholesterol bilayers, ca. 2.8 nm).[23,25] The solid-state structure of a shorter foldamer with a hydroxyl C-terminus, Aib₃Aib₅CH₂OH 7 (Figure 2b), also shows a 3₁₀-helical core (view down axis, Figure 2c) with an end-to-end distance of 1.87 nm. The C-terminal hydroxyl forms a strong hydrogen bond with the preceding Aib residue (H to O distance 1.868 Å, Figure 2b,c) instead of hydrogen bonding to the carbonyl of the i + 3 Aib residue, which would continue the 3₁₀ helix (H to O distances in the 3₁₀-helix backbone typically 2.1 to 2.2 Å). This is in contrast to N-terminal acylation of Aib foldamers, which adds a hydrogen bond (from the amide carbonyl) that supports the 3₁₀-helical structure, as shown in the structure of AcAib₅OBU.[26]

8-Hydroxypyrene-1,3,6-trisulfonate (HPTS) assays

The HPTS assay is widely used to assess the ionophoric ability of compounds.[27] The HPTS dye is a ratiometrically sensitive probe that can report on the discharge of a pH gradient established between the exterior and interior of phospholipid vesicles. The discharge of the gradient can occur via H⁺/M⁺ antiport or H⁺/X⁻ symport. The measured rate of change of the pH reflects a number of molecular level events, including the rate of membrane insertion as well as the conductance induced by the compounds.[27]

HPTS assays were carried out on compounds 1-15 using procedures developed previously.[25] Aliquots of foldamers in methanol were equilibrated for 180 s with suspensions of large unilamellar vesicles (composed of 4:1 egg yolk phosphatidylcholine:cholesterol mol/mol, 800 nm diameter) in MOPS buffer (20 mM MOPS, 100 mM NaCl, pH 7.4). Then a NaOH base pulse was used to change the external pH to 8.4 and the subsequent change in HPTS fluorescence was measured. The addition of Triton X-100 27 minutes later lysed the vesicles and provided the maximum change in HPTS fluorescence for data normalisation.

As found previously,[23] the ability of Aib foldamers to discharge a pH gradient showed a clear length dependence (Figure 3). The fractional discharge of the ion gradient after 1000 s (normalised against Triton X-100 addition) can give a measure of the activity of each compound. By this measure, each incremental addition of an Aib₂ unit to homologous foldamers gives an order of magnitude increase in activity, i.e. similar HPTS activity to 10-fold higher concentrations of the shorter homologues (see the ESI).

Upon comparing the effect of different termini within a family of foldamers of given length, a pattern emerges. These compounds with the hydrophobic TIPS group at the C-terminus
show consistently high activity, which appears to be altered little by an acetyl group at the N-terminus. Polar modifications at the C-terminus seem to be detrimental to activity, with the alcohols showing lower activity and the acids (predominantly present as the carboxylates) showing very low activity even at a seven-fold greater concentration (Figure 3c). This loss of activity for foldamer 9 mirrors the loss of activity reported for trichogin GA IV analogues that have a C-terminal carboxylate, an effect ascribed to electrostatic repulsion with the lipid phosphate groups.\textsuperscript{[18a]} The one foldamer with a C-terminal primary amide that was tested (N\textsubscript{2}Alb\textsubscript{2}C(O)NH\textsubscript{2}) also showed a decrease in ionophoric activity compared to a TIPS. Only in the Alb\textsubscript{2} series of compounds did a C-terminal alcohol show high activity, albeit still lower that the TIPS protected analogue. These longer foldamers with non-polar termini had activity in these HPTS assays that was around 70\% of that shown by alamethicin (Figure 3c).

These observations suggest that the length of the foldamer and the ability to partition into the hydrophobic part of the bilayer are the most import factors in determining ionophoric activity.

**Planar bilayer conductance assays**

Planar bilayer conductance (PBC) data can provide information on the mechanism by which ionophoric compounds conduct ions across membranes; the appearance of discrete conductance events can indicate the formation of channels and provide information on their behaviour. Alamethicin has been well studied by PBC, producing discrete conductance events that are thought to be due to “barrel-stave” multimeric channels comprising 3-12 helical monomers. The number of peptide units in a channel is dynamic, producing multiple conductance levels that correspond to channels of different molecularity.\textsuperscript{[60-62]} Alamethicin also shows non-ohmic behaviour, with high positive potentials (+100 mV, 10 nm peptide) producing bursts of discrete conducting states ranging from 0.52 to 5.0 nS that arise from relatively large conductance “barrel-stave” channels.\textsuperscript{[20]}

Alb foldamers with hydrophobic termini have been shown to have PBC behaviour similar to that of alamethicin. Incrementally longer foldamers (N\textsubscript{2}Alb\textsubscript{1}C(O)OCH\textsubscript{2}CH\textsubscript{2}TMS, \textit{n} = 7, 8, 11, 13) were studied by PBC in EYPC/cholesterol 4:1 bilayers.\textsuperscript{[23]} At ≤5 \textmu m the shortest two foldamers produced irregular and “spike” conductances (using terminology proposed by Chiu and Fyles\textsuperscript{[29]}, indicating transient membrane disruption, and at ≥10 \textmu m they caused the membranes to break. The longer compounds did not weaken the membrane in the same way, with “multi-level” and “flicker” behaviour observed. The “open” states typically lasted <0.2 s, and were of varied conductance between 100 and 5000 pS. Quantised steps in the conductance levels were not observed, suggesting that multiple openings of the same type of pore did not occur.

Foldamers with different lengths that also showed good ionophoric activity in the HPTS assays were selected for PBC assays. AcAlb\textsubscript{2}CH\textsubscript{2}OTIPS 6, AcAlb\textsubscript{1}CH\textsubscript{2}OH 8, N\textsubscript{2}Alb\textsubscript{2}C(O)NH\textsubscript{2} 10, N\textsubscript{2}Alb\textsubscript{1}CH\textsubscript{2}OTIPS 11, AcAlb\textsubscript{1}CH\textsubscript{2}OTIPS 12, N\textsubscript{2}Alb\textsubscript{1}CH\textsubscript{2}OH 13, AcAlb\textsubscript{1}CH\textsubscript{2}OH 14. At 16 \textmu m none of these compounds caused the membranes to break, but they showed clear differences in conductance behaviour.

Discrete conductance events were only observed for compounds with N-acetyl termini, whereas foldamers 8, 11 and 13 showed few or no conductance events within 7 h of addition to the membrane. N\textsubscript{2}Alb\textsubscript{2}C(O)NH\textsubscript{2} 10 was mostly inactive or with low level conductance. However three of the four acetylated compounds gave regular “square-top” activity, which lasted up to 500 ms for 6 and 12 and several minutes for 14, along with flickering and erratic conductances (Figure 4).

![Figure 4](image)

**Figure 4.** a) Examples of planar bilayer conductance for 6, 12 and 14, all 16 \textmu m. Applied voltages indicated for each trace, zero current position by horizontal line, arrow indicates size and direction of current. (a) 6 (AcAlb\textsubscript{2}CH\textsubscript{2}OTIPS), max current passed +9 pA. (b) 6, max. current passed +34 pA. (c) 12 (AcAlb\textsubscript{1}CH\textsubscript{2}OTIPS), steps of +25, +76 and +101 pA from baseline; maximum may be due to four open pores. (d) 12, current passed for smaller steps +17 pA. (e) 14 (AcAlb\textsubscript{1}CH\textsubscript{2}OH), max. current passed between closed and open states shown, step size -34 pA, max. current passed -48 pA. (f) 14, max. current passed -23 pA.

In contrast to inactive AcAlb\textsubscript{2}CH\textsubscript{2}OH 8, AcAlb\textsubscript{1}CH\textsubscript{2}OTIPS 6 showed some conductance behaviour although this was not as consistent as the longer peptides. “Multilevel” activity (Figure 4a)
was observed as well as “square-top” activity arising from stable pores with on/off activity at similar current levels (up to 34 pA at +100 mV, Figure 4b). AcAib$_2$CH$_2$OTIPS 12 gave “square-top” conductance events within 20 minutes (Figure 4c), with spiky traces also frequently observed (Figure 4d). The “square-top” events suggested channels with the same level of conductance were formed, which opened and closed much more frequently (open channel lifetimes were typically <100 ms) than those observed for AcAib$_1$CH$_2$OH 14. Foldamer 14 gave conductance events more slowly than 12, but around 2 h after addition of 14 to the cis well, a stable pore opened (Figure 4e) following a period of spiky conductance and ill-defined pore formation (Figure 4f). For both 12 and 14, it may be that the transient conductance events are due to incomplete channel formation. Notably, the same conductance was measured in multiple, repeat experiments, indicating this compound reproducibly forms a pore of the same dimensions, rather than poorly-defined membrane weakening. This reproducible quantised membrane activity indicates ion channel formation with the same molecularity for each pore. The lifetime and magnitude of the “open” conductance state of these channels (ca. 300 pS over 500 ms to several seconds) were distinct from typically reported square-topped traces (10–30 pS conductance over 1–10 s durations), but similar to those seen for N$_2$Aib$_2$C(O)OCH$_2$CH$_2$TMS (n = 11 or 13). Comparing the behaviour of 12 and 14 suggests that a C-terminal alcohol may stabilise the open state of the pore, resulting in the observed quantised channel opening/gating behaviour.

These PBC studies indicate that the presence of an N-terminal acetyl group correlates with the observation of conductance events arising from discrete channels. An N-terminal acetyl group is known to stabilise folding, increase the end-to-end dipole, and strongly promote self-association of Aib foldamers in hydrophobic environments, all factors that should promote ion channel formation. In contrast a C-terminal hydroxyl group did not produce a marked increase in conductance events in the PBC assays, even though the duration of pore opening for AcAib$_1$CH$_2$OH 14 was significantly longer than for the other foldamers. A C-terminal TIPS group did improve PBC activity in one case (e.g. 6 vs. 8), although an N-terminal acetyl group was also present. It may be that the lack of a distinct effect occurs because the terminal hydroxyl of the reduced Aib forms an i → i+1 hydrogen bond (as found in 7) instead of an i → i+3 hydrogen bond that continues, and potentially stabilises, the membrane-spanning 3i0-helix. It is interesting to note that in natural peptibols the reduced C-terminal residue is never an Aib.

Clear channel forming behaviour in the PBC studies did not always correlate with good ionophoric activity in HPTS assays. Some foldamers that showed long-lived, stable pores in single channel studies (e.g. 12 and 14) had lower HPTS activity than analogous N-terminal azides that showed no activity in the PBC assays (e.g. 11 and 13 showed no activity within 7 h of addition). It is possible that the latter may form channels with lifetimes too brief to be observed as discrete events by PBC (i.e. μs rather than ms durations). This would result in a small, but constant ion current leakage across the bilayer that is difficult to distinguish from drift in the electrode potentials, or background leakage of the bilayer over the duration of the experiments. Alternatively, there may be a different mechanism of action under an applied voltage that favours channel formation. For example, an external field may induce transmembrane alignment of compounds, such as peptides, which have strong end-to-end dipoles that increase with helical length. Furthermore, the voltage-induced flow of ions through a channel may help to maintain open channels. Indeed, PBC measurements are performed under out-of-equilibrium conditions (constant transmembrane potential gradient), whereas the HPTS assay is approaching equilibrium over time (dissipating a transmembrane ion gradient).

Antibacterial activity assays

It has been proposed that the antibiotic activity exhibited by alamethicin against yeast and Gram-positive bacterial strains arises from the formation of high-conductance channels in cellular membranes. To assess the antibiotic activity of these foldamers, simple and qualitative measure can be obtained using an agar diffusion assay. After plating of the bacterial strain onto the dish, a methanol solution of the compound of interest is evaporated at a point on an agar plate, allowing the compound to diffuse into the agar gel. After incubation for a set time period, a zone of growth inhibition (Figure 5a) occurs where the compound has prevented bacterial growth.

Foldamers N$_2$Aib$_2$CH$_2$OH 7, AcAib$_2$CH$_2$OTIPS 6, AcAib$_1$CH$_2$OH 14 and N$_2$Aib$_1$CH$_2$OTIPS 11 were selected for antibiotic assays against Bacillus megaterium strain DSM319 as they had shown either good ionophoric activity in the HPTS assay or discrete conductance events in the PBC assays. Alamethicin, which has good activity against B. megaterium, was used as a reference compound, along with 16, 17 and 18, which had been previously assessed for ionophoric activity (HPTS assays for 16, 17, 18; PBC assay for 18).

After addition of 0.4 nmol compound (1 μL of 400 μM stock, mass from 0.3 μg for 7 to 0.46 μg for 11) then plating of B. megaterium strain DSM319 onto the Petri dish (150 mm diameter), the resulting diameter of the inhibition zone (IZ) was measured after incubation at 30 °C for 16-18 h. Clear differences were observed between compounds with different C-termini. The TIPS/TMS terminated compounds all showed moderate (6) or good activity (11, 17 and 18), yet the hydroxyl terminated compounds (7 and 14) showed no activity above the methanol control. The longer compounds 11, 17, 18 with hydrophobic termini showed activity that was comparable to that of alamethicin (0.4 nmol, 0.8 μg). Doubling the amounts on the agar to 0.8 nmol increased the size of the inhibition zone for 14 (IZ = 0.41 ± 0.06 cm, 11, 17, 18 all have IZ > 1 cm), but not for shorter foldamer 7 (IZ = 0.08 ± 0.04 cm); both are inactive relative to methanol (see ESI). This antibacterial activity was not caused by the Aib residue itself, as the tetrameric foldamer N$_2$Aib$_2$C(O)OCH$_2$CH$_2$TMS and hexameric foldamer 16 showed very low activity even at 1 nmol (0.48 μg and 0.63 μg). Much like alamethicin, none of the foldamers examined showed activity against Gram-negative E. coli.
foldamer length beyond ten Aib residues, and hence forming a 3_{10} helix longer than that needed to span the hydrophobic core, did not seem to produce a significant increase in antibacterial activity. Increasing the length of the foldamer core beyond Aib_{10} does however lead to sharply lower solubility in organic solvents.

Conclusions

An N-terminal acetyl group can allow Aib foldamers to form long-lived and well-defined channels in planar bilayers, and did not in general adversely affect ionophoric performance in vesicles or antibacterial behaviour. Although it does not produce a significant improvement in activity in either vesicle or cell assays compared to an N-terminal azide, we suggest that a role of N-terminal acyl groups in peptaibols is to mask the basic amine and make this terminus less hydrophilic. Indeed it has been suggested that peptaibols have N-terminal acyl groups and C-terminal 1,2-aminoalcohols, simply to remove the respective positive and negative charges from their backbones.\[35] We also propose that an N-terminal acetyl group plays a role in stabilising the helical secondary structure required to span the membrane by extending the hydrogen-bonding network. Furthermore the lack of PBC activity for otherwise very active N-terminal azide foldamers suggests that solely using patch-clamp methods to screen for membrane activity may result in promising lead compounds for antibiotics being overlooked.

In contrast, a C-terminal hydroxyl group mildly inhibited activity in the vesicle assays and significantly diminished antibacterial activity compared to C-terminal esters. Given reports that C-terminal esters of peptaibolin are as membrane active as the corresponding alcohols,\[16b] and that the membrane and antibacterial activities of C-terminal ester analogues of trichogin GA IV are either comparable\[19] or slightly weaker\[18a] than the parent compound, it may be that a C-terminal hydroxyl functions as a hydrolytically stable modification with lower polarity than a carboxylate. It is interesting to note that recent studies have indicated that, when compared to the C-terminal methyl ester analogue, the C-terminal alcohol in trichogin GA IV leads to significantly higher toxicity against human cell lines but not bacteria.\[18a] However our studies do not pinpoint the role played by the C-terminal hydroxyl in the antibiotic activity of peptaibols, as the natural compounds do not have a reduced Aib at the C-terminus.\[35] Perhaps a gem-dimethyl stabilises a i→i+1 hydrogen bond (as observed in 7) over other arrangements that facilitate channel formation.

Synthetic foldamers that mimic the behaviour of the peptaibols could lead to new antibiotics bearing bioactive motifs that are not biologically accessible. Our studies indicate there is substantial scope for modifying the termini of an Aib foldamer core, which may even provide synthetic peptaibols with improved selectivity for bacterial over mammalian cells.

These data from the agar diffusion assays are only qualitative, but indicate that the structure-activity relationship for antibacterial activity corresponds best with that found for the HPTS assay, with compound length (i.e. 17, 18 > 16) and C-terminal lipophilicity (i.e. 6 > 7) playing key roles. Unfortunately the effect of an N-terminal acetyl group on antibacterial activity could not be conclusively identified due to the limited range of compounds studied. However, the existence of lipopeptoids,\[2] which combine a short peptide component with a N-terminal acyl chain (C8 to C15), suggests that N-terminal lipophilicity can also be beneficial. In line with previous results,\[23] increasing the

Figure 5. (a) Photograph of agar plate with MeOH and foldamers (each 0.4 and 0.8 nmol) added; AcAib_{12}CH_{2}OTIPS 6, N_{2}Aib_{12}CH_{2}OH 7, N_{2}Aib_{12}CH_{2}OTIPS 11, AcAib_{11}CH_{2}OH 14, N_{2}Aib_{11}CH_{2}OH \[17], N_{2}Aib_{10}TMS 18, and alamethicin. Scale bar 1 cm. (b) Inhibition zone diameters against B. megaterium strain DSM319 (cm, mean of 12 measurements) for foldamers 6, 7, 11, 14, 17, 18 and N_{2}Aib_{10}TMS 16. Each foldamer is 0.4 nmol except Aib hexamer 16 (1.0 nmol). Standard errors of the mean are shown.
Experimental Section

The synthetic procedures for the preparation of new foldamers 1 to 15 are reported in the Supporting Information, along with corresponding $^1$H NMR and $^{13}$C NMR spectra. Foldamers 16 to 18 were prepared according to literature procedures. X-ray crystallography details are reported in the Supporting Information. Structural data for 7 and 11 have been deposited with the Cambridge Crystallographic Data Centre. CCDC 1582012 and 1582013 contain the supplementary crystallographic data for 7 and 11 respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request. Procedures for HPTS, PBC and antibacterial assays are also reported in the Supporting Information.

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[33] The time between compound addition to the plate and inoculation with bacteria has been suggested to be important due to the relatively slow diffusion time for peptaibols from the agar. See reference [10a].
Cap it off: To uncover how the structural features of peptaibols affect their membrane activity, α-aminoisobutyric acid (Aib) foldamers with different lengths and terminal groups were synthesised. The longest foldamers (eleven residues) with hydrophobic termini had the highest ionophoric activity in vesicles and best antibacterial activity, but only N-acetylated foldamers produced long-lived discrete ion channels.