Synthesis of a chiral amino acid with bicyclo[1.1.1]pentane moiety and its incorporation into linear and cyclic antimicrobial peptides†

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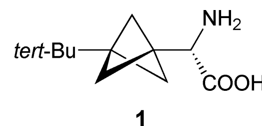
The synthesis of the lipophilic chiral amino acid **1** bearing the bicyclo[1.1.1]pentane moiety is described. Linear and cyclic hexapeptides of the type Arg-Arg-Xaa-Yaa-Arg-Phe containing **1** instead of one or two tryptophan residues are prepared by solid phase peptide synthesis and the antimicrobial and hemolytic activity of the peptides obtained are discussed.

Introduction

Antimicrobial peptides are important components of the animal defence against microbial infections.[1] Stimulated by the challenge of increasing bacterial resistance, considerable efforts are being made to elucidate the structural basis of peptide selectivity and their mechanism of action. It is generally accepted that the peptides exert their activity by permeabilisation of the lipid matrix of the bacterial membrane. Peptide interaction with the negatively-charged lipid matrix of prokaryotic cells is the consequence of several common structural features of the peptides such as cationic charge and the tendency to adopt an amphipathic conformation in the membrane-bound state. Because of their activity against a broad spectrum of pathogens, but low activity against normal eukaryotic cells and their unique mode of action, the peptides have been heralded as new antibiotic drug candidates. But, despite their attractive properties, and many efforts to optimise the peptide properties with respect to high antibacterial activity and selectivity, issues regarding toxicity and instability are unresolved and the high production costs of the large peptides render additional concerns. It is thus important to develop smaller antibacterial peptides with adequate metabolic stability. There are only a few examples of antibacterial peptides of small size in the literature. A six-residue sequence was identified as the antimicrobial motif of bovine lactoferricin[2] and *N*-acetylated hexapeptides such as Ac-RRWWRF-NH_2 have been identified by deconvolution of combinatorial libraries.[3] A recent study to define the minimum requirements of charge and lipophilic properties in antimicrobial peptides led to even shorter sequences with a remarkable antimicrobial effect.[4] All these peptides are rich in arginine and tryptophan residues which play an important role in their activity. Studies with analogues modified in the position of the aromatic residue have demonstrated the significance of the size, shape and character of the side chain for the antimicrobial effect.[5–7] Our recent studies showed that cyclisation of RRWWRF distinctly enhances the antimicrobial activity by inducing a constrained amphipathic structure, and additionally increases resistance against proteolysis.[7,8] The effect of cyclisation on the enhancement of the antimicrobial activity is especially pronounced for the tryptophan-containing sequences. The substitution of tryptophan by the more lipophilic 2-naphthylalalanine (2-Nal) increases the antimicrobial effect of the linear sequences, and transformation into the cyclic analogues does not result in further improvements.[6,7] Remarkably, the insertion of the lipophilic, aromatic Nal residues increases also the lysis of erythrocytes,[7] thus making these peptides less suitable as potential antimicrobial reagents.

In order to study the influence of lipophilic, non-aromatic amino acid side chains on the antimicrobial and hemolytic activity, we synthesised the corresponding linear and cyclic peptides, containing amino acids derived from the bicyclo[1.1.1]pentane moiety. The chemistry of bicyclo[1.1.1]pentanes has gained considerable attention over the past years and a broad range of differently substituted derivatives has been synthesised.[9] Among them 2-(3′-substituted bicyclo[1.1.1]pentyl)glycines are the only examples of α-amino acids bearing the bicyclo[1.1.1]pentane moiety.[10] These compounds represent selective metabotropic glutamate 1 receptor (mGluR) antagonists, with no activity at other mGluR subtypes. Recently, the synthesis of the γ-amino acid 3-aminobicyclo[1.1.1]pentane-1-carboxylic acid and its incorporation into peptides has been reported.[11]

Here we report on the synthesis of the novel amino acid **1** bearing the bicyclo[1.1.1]pentane moiety, the incorporation of **1** into peptides of the type Arg-Arg-Xaa-Yaa-Arg-Phe, and the antimicrobial and hemolytic activity of both the linear and cyclic analogues.



Results and discussion

The synthesis of target compound **1** is depicted in Scheme 1. Treatment of the dibromocyclopropane **2** with 2.2 equivalents of methyllithium generated the [1.1.1]propellane **3**.[12] Addition of *tert*-butylmagnesium chloride to the central bond of **3** afforded a

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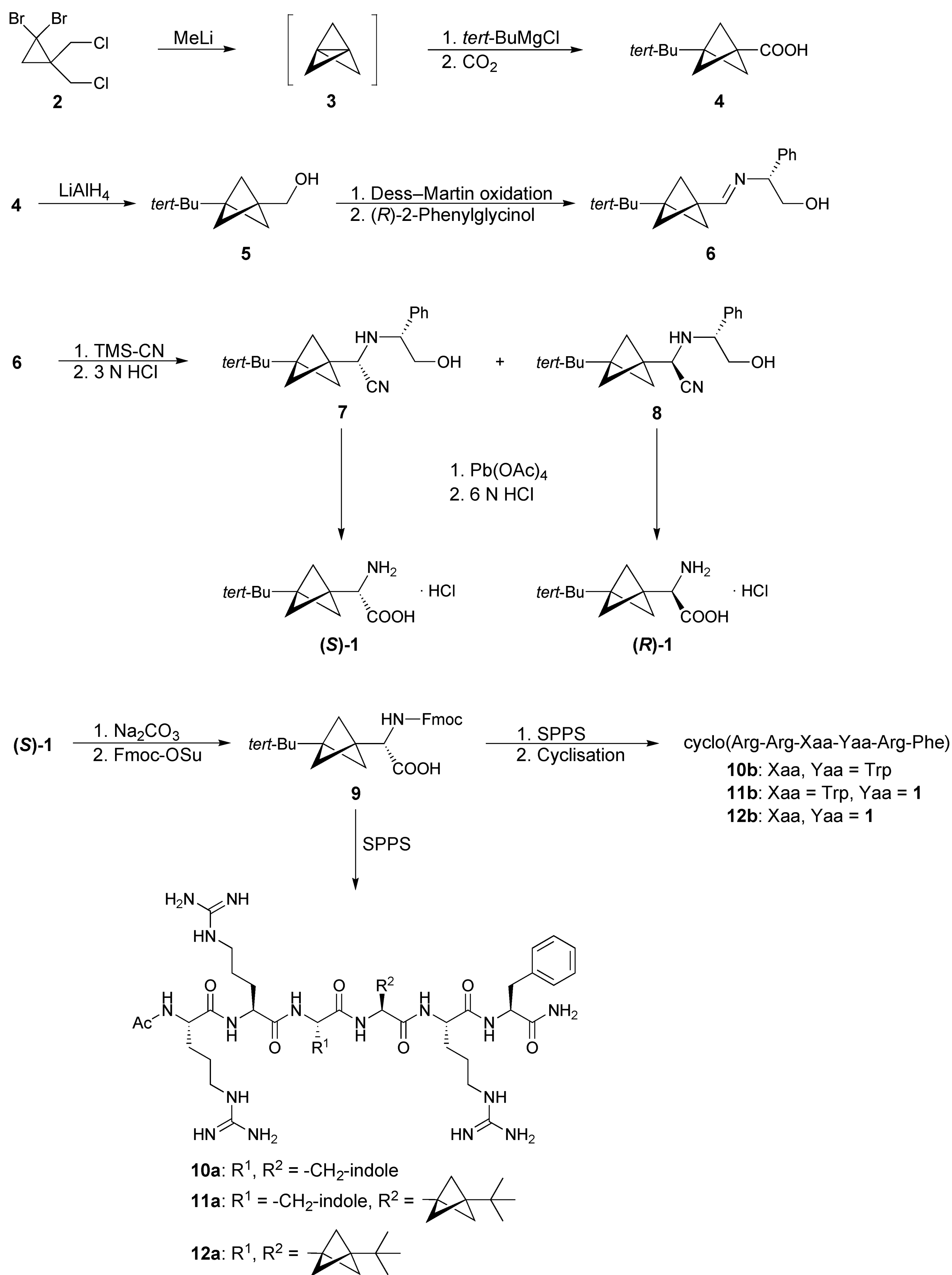

Scheme 1 Synthesis of amino acid **1** and peptides containing **1**.

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3-*tert*-butylbicyclo[1.1.1]pentyl magnesium chloride, which could be trapped by carbon dioxide to give acid **4**. This compound was reduced to the alcohol **5** by lithium aluminium hydride and subsequently converted to the corresponding aldehyde *via* a Dess–Martin-oxidation. Condensation of the aldehyde with (*R*)-2-phenylglycinol gave imine **6**, which was subjected to an asymmetric Strecker reaction.[10,13] The 7/3-mixture of diastereomeric α-aminonitriles **7** and **8** was separated by flash chromatography. Finally, the auxiliary was removed oxidatively and the nitrile hydrolysed to give the amino acid **1**.[10,13] Treatment of (*S*)-**1** with base and Fmoc-protection resulted in the formation of compound **9** which is a suitable derivative for solid phase peptide synthesis possessing good solubility in DMF.

Linear and cyclic analogues of the hexapeptide sequence RRWWRF (**10a**) containing **1** instead of one or two tryptophan residues were synthesised manually by standard Fmoc-SPPS techniques. Rink amide and 2-chlorotrityl resins were used for the linear peptides and for the linear precursors of the cyclic peptides, respectively. The linear peptide amides (**10a**–**12a**) were N-terminally acetylated. Cyclisation was achieved by activation with HAPyU giving the cyclic analogues **10b**–**12b**. According to HPLC, purity of linear and cyclic peptides was >95 and >85%, respectively. Molecular masses of all peptides were confirmed by MALDI mass spectrometry (see the ESI† for synthesis, purification and analytical data of the peptides **10a/b**–**12a/b**).

Small, arginine- and tryptophan-rich hexapeptides such as Ac-RRWWRF-NH_2 (**10a**) have been found to possess antimicrobial activity.[3] Our recent studies showed that cyclisation of RRWWRF markedly enhances the antimicrobial effect[7] by inducing an amphipathic structure.[8] Although the influence of the side chains upon the structure of the cyclic peptides in a membrane-mimicking environment was low, substitution of the aromatic side chains by bulky residues of varying hydrophobicity and size had dramatic consequences on the biological effect.

To further elucidate the activity-modifying character of side chains, linear and cyclic hexapeptides were synthesised with the novel aliphatic, highly hydrophobic amino acid **1** substituting one or two of the tryptophan residues, respectively (Table 1).

The gradual changes in the retention times of the linear peptides which followed the order **10a** < **11a** < **12a** reflected changes in the total hydrophobicity and/or amphipathicity as result of amino acid exchange (Table 1). Comparable retention behaviour of the corresponding cyclic compounds suggests that conformational constraints have little influence on the surface behaviour. These properties are also reflected in the peptide interactions with lipid bilayers. With introduction of **1**, the bilayer-disturbing activity distinctly increased, but pronounced differences between linear and cyclic analogues were not observed (Fig. 1B).

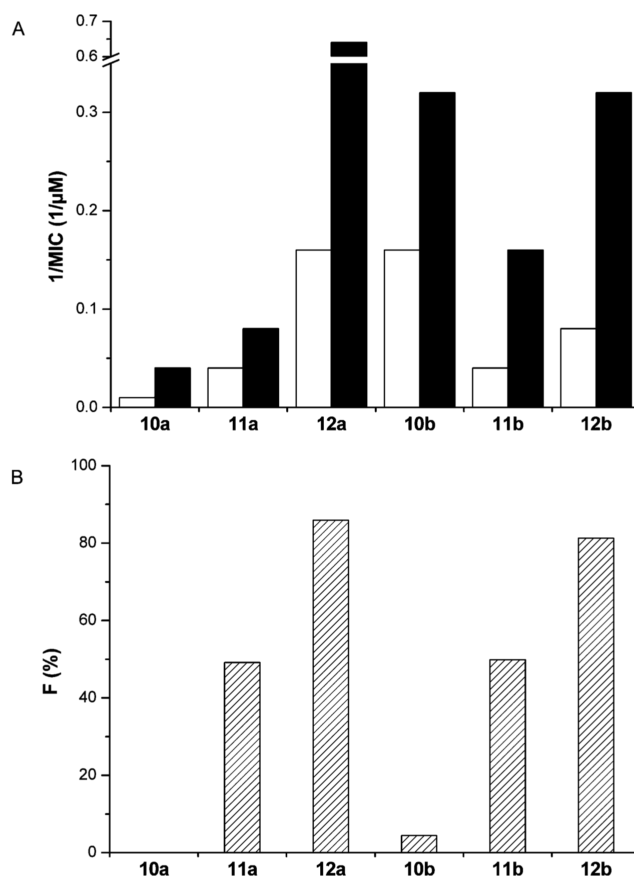

Fig. 1 (A) Reciprocal minimal inhibitory concentration (1/MIC) of linear and cyclic peptides against *E. coli* and *B. subtilis* (white and black bars); (B) peptide-induced dye release from mixed POPG–POPC (1 : 3) vesicles (striped bars) measured as fluorescence dequenching (F) at $c_{peptide}$ = 2 μM and c_{lipid} = 25 μM after t = 5 min.

The linear peptide **10a** had little activity against bacteria, as shown by a minimal inhibitory concentration (MIC) higher than 100 μM (1/MIC < 0.01 μM^{-1}) for *E. coli* (Gram-negative). *B. subtilis* (Gram-positive) were slightly more susceptible (1/MIC = 0.04 μM^{-1}) (Fig. 1A). With introduction of one or two of the bulky, highly hydrophobic residues **1**, the antimicrobial effect of the linear sequence markedly increased. These results are similar to effects obtained by introduction of bulky aromatic residues into lactoferricin[14] and confirm the important role of peptide hydrophobicity for the activity of the hexapeptides.[7]

In contrast to the pronounced enhancement of the antimicrobial activity by cyclisation of the parent peptide **10a** (Fig. 1A), the positive effect of cyclisation disappeared for the analogues

Table 1 Sequences and retention times of linear and cyclic peptides

Linear			Cyclic		
Name	Sequence	t_R/min[a]	Name	Sequence	t_R/min[a]
10a	Ac-RRWWRF-NH_2	17.6	**10b**	Cyclo(RRWWRF)	18.5
11a	Ac-RRW**1**RF-NH_2	20.1	**11b**	Cyclo(RRW**1**RF)	19.9
12a	Ac-RR**11**RF-NH_2	23.0	**12b**	Cyclo(RR**11**RF)	23.9

[a] t_R: retention time in RP-HPLC.

substituted by **1**. Whereas **10b** exhibited a more than 16-fold enhanced activity against *E. coli* and eight times higher activity against *B. subtilis* than its linear counterpart, the activities of the linear and corresponding cyclic peptides containing one residue **1** (**11a/b**) were almost the same, while the activity of **12b** decreases significantly when compared to **12a**. Furthermore, different to the observations in HPLC (Table 1) and the bilayer permeabilisation assay (Fig. 1B), the biological activities of all cyclic peptides were little differentiated. As suggested for the naphthylalanine-containing hexapeptide, it is likely that the high global hydrophobicity of the sequences containing **1** eliminated the activity-enhancing effect of cyclisation-induced amphipathicity.[7] Obviously, aromatic residues are not essential. Furthermore, the different activity patterns on model membranes and bacteria show that peptide interactions with the biological membrane are highly complex, and hydrophobic peptide interactions with the lipid matrix of the cell membrane are not sufficient to explain the activity towards bacterial cell membranes.

In summary, our studies showed that substitution of tryptophan by the highly hydrophobic and bulky residues **1** resulted in peptides with pronounced antimicrobial activity. Comparable to the aromatic naphthylalanine, the high hydrophobicity of **1** predominated peptide membrane interactions and the activity-enhancing effect of cyclisation, observed for the tryptophan-containing peptide, became negligible.

Conclusion

Synthesis of the chiral amino acid **1** was achieved in nine steps from the tetrahalide **2**. Key steps in the synthesis were the Grignard-addition to [1.1.1]propellane **3** and the asymmetric Strecker-reaction yielding the two glycinonitriles **7** and **8** in a diastereomeric ratio of 7 : 3. After Fmoc-protection of **1**, several linear and cyclic peptides containing this amino acid could be synthesised by SPPS and their antimicrobial activity was determined. Substitution of tryptophan by **1** in the linear peptide RRWWRF (**10a**) enhances both the antimicrobial and bilayer-permeabilising activity. To the same extent, hydrophobicity increases in these compounds. Thus, the higher activity of **11a** and **12a** can be attributed to a higher membrane-disturbing effect due to higher hydrophobicity. Cyclisation of **11** and **12** has only minor influence on bioactivity. On the other hand, the antimicrobial activity of **10b** is distinctly enhanced upon cyclisation, while lysis remains low.

Experimental

All experiments were carried out under a nitrogen atmosphere. Solvents were dried according to standard procedures before being used. *t*BuMgCl was purchased from Aldrich Co. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker DPX 300. Mass spectra were obtained on a ThermoFinnigan LCQ XP. Elemental analyses were performed on a Leco CHNS-932 apparatus. Optical rotations were measured on a Jasco DIP-370 polarimeter at 589 nm and are given in 10^{-1} deg cm^{2} g^{-1}.

3-*tert*-Butylbicyclo[1.1.1]pentane-1-carboxylic acid (4)

MeLi (225 ml of a 1.2 M solution in diethyl ether, 0.27 mol, 2.00 equiv.) was added dropwise to a solution of 2,2-bis(chloromethyl)cyclopropane **2** (40 g, 0.135 mol, 1.00 equiv.) in diethyl ether (150 ml), which was kept at −30 °C under stirring. The mixture was stirred for 1 h at room temperature, until the formation of a white precipitate was complete. The solvent and the volatile products were distilled at 14 Torr from a 20 °C bath into a Schlenk flask which was kept at −78 °C.[12] To the obtained clear solution was added dropwise *tert*-butylmagnesium chloride (47 ml of 2.0 M solution in diethyl ether, 0.094 mol, 0.70 equiv.) at −50 °C. The reaction mixture was stirred for 4 d at room temperature. Then CO_2 (from 40 g dry ice, dried over silica gel) was passed into the grey–green reaction mixture for 2 h at −40 °C. The reaction was allowed to warm to room temperature and then cooled to 0 °C. Aqueous HCl (100 ml, 2 N) was added at this temperature. The organic phase was separated and the aqueous layer was extracted with diethyl ether (3 × 25 ml). Drying the combined organic layers over $MgSO_4$ and evaporation of the solvent afforded carboxylic acid **4** (14.0 g, 61% from **2**) as colourless crystals. Mp: 167 °C (lit.[15]: 155–157 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 9 H), 1.86 (s, 6 H), 10.54 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 29.5, 35.5, 48.2, 48.3, 177.4. The NMR data correspond to the literature.[15]

3-*tert*-Butylbicyclo[1.1.1]pent-1-ylmethanol (5)

To a suspension of $LiAlH_4$ (11.4 g, 0.3 mol) in THF (250 ml) carboxylic acid **4** (20.43 g, 0.121 mol), dissolved in THF (110 ml) was added dropwise over 30 min at 0 °C. The mixture was heated under reflux for 1 h. After cooling to 0 °C diethyl ether (300 ml) and a sat. aq. solution of $MgSO_4$ (40 ml) were added. The suspension was filtrated through a pad of celite. The filtrate was extracted with diethyl ether (3 × 50 ml). Drying the combined organic layers over $MgSO_4$ and evaporation of the solvent afforded the target compound **5** (15.98 g, 85%) as colourless crystals. Mp: 40–41 °C (lit.[16]: 46–47 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (s, 9 H), 1.28 (s, 1 H, 1.48 (s, 6 H), 3.59 (s, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 29.7, 37.2, 45.0, 48.4, 63.9. The NMR data correspond to the literature.[16]

3-*tert*-Butylbicyclo[1.1.1]pentane-1-carbaldehyde

To a solution of 3-*tert*-butylbicyclo[1.1.1]pentane-1-methanol **5** (10.08 g, 65.35 mmol) in CH_2Cl_2 (200 ml) was added Dess–Martin periodinane[17] (27.72 g, 65.35 mmol) at 0 °C within 10 min. After stirring for 2 h a solution of $Na_2S_2O_3 \times 5\ H_2O$ (100 g) in sat. aq. $NaHCO_3$ (400 ml) and 200 ml diethyl ether were added carefully to the white suspension formed. After stirring for 1.5 h the layers were separated and the aqueous phase was extracted with diethyl ether (3 × 80 ml). Drying the combined organic phases over $MgSO_4$ and evaporation of the solvents afforded the target aldehyde (9.64 g, 97%) as pale yellow oil. The aldehyde was used in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 9 H), 1.81 (s, 6 H), 9.59 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.8, 44.9, 45.9, 46.9, 48.7, 200.1.

(2*S*,1′*R*)- and (2*R*,1′*R*)-2-(3-*tert*-Butylbicyclo[1.1.1]pent-1-yl)-*N*-(2′-hydroxy-1′-phenylethyl)glycinonitrile (7 and 8)

To a solution of 3-*tert*-butylbicyclo[1.1.1]pentane-1-carbaldehyde (3.08 g, 20.2 mmol) in CH_2Cl_2 (175 ml) was added (*R*)-2-phenylglycinol[18] (2.78 g, 20.3 mmol) within 5 min. The solution was stirred at room temperature for 16 h. The reaction mixture was cooled to −65 °C and trimethylsilylcyanide (5.07 ml, 4.02 g,

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40.5 mmol) was added *via* a syringe. The reaction mixture was allowed to warm up slowly to room temperature. After 20 h the solution was cooled to 0 °C and hydrochloric acid (100 ml of 3 N aq. solution) was added *via* a dropping funnel and the mixture was stirred for 1 h. The layers were separated and the organic phase washed with hydrochloric acid (30 ml of 3 N aq. solution). The combined aqueous layers were extracted with CH_2Cl_2. The combined organic phases were washed with H_2O, and dried over $MgSO_4$. The solvent was removed under reduced pressure to afford a yellow oil (5.00 g), which was subjected to column chromatography (pentane–ethyl acetate = 8 : 2) to give the pure (2*S*,1′*R*)-derivative **7** (1.86 g, 30% from the aldehyde, colourless crystals) and a mixture (1.22 g, 20%) of **7** and diastereomer **8**. The mixture was subjected to a second chromatography with the next reaction batch. Compound **7**: mp: 54–57 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 9 H), 1.54–1.68 (m, 6 H), 2.12 (br s, 2 H), 3.37 (s, 1 H), 3.56 (d × d, $J = 9.0$ and 10.9 Hz, 1 H), 3.78 (d × d, $J = 3.8$ and 10.9 Hz, 1 H), 4.08 (d × d, $J = 4.1$ and 9.0 Hz, 1 H), 7.24–7.41 (m, 5 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 29.6, 36.4, 45.1, 48.2, 49.7, 63.1, 67.6, 118.8, 127.7, 128.3, 128.9, 138.5. Found: C, 76.11; H, 8.98; N, 9.19. $C_{19}H_{26}N_2O$ requires C, 76.47; H, 8.78; N, 9.39%. Compound **8**: yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (s, 9 H), 1.55–1.68 (m, 6 H), 2.03 (br s, 2 H), 3.64 (d × d, $J = 7.2$ and 10.9 Hz, 1 H), 3.70 (s, 1 H), 3.75 (d × d, $J = 4.5$ and 10.9 Hz, 1 H), 3.99 (d × d, $J = 4.3$ and 7.3 Hz, 1 H), 7.28–7.41 (m, 5 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 29.6, 36.8, 45.3, 48.2, 50.2, 63.2, 66.3, 118.8, 127.6, 128.4, 129.0, 139.6. Found: C, 76.18; H, 8.93; N, 9.26. $C_{19}H_{26}N_2O$ requires C, 76.47; H, 8.78; N, 9.39%.

(*S*)-2-(3-*tert*-Butylbicyclo[1.1.1]pent-1-yl)glycine hydrochloride [(*S*)-1]

To a solution of glycinonitrile **7** (1.73 g, 5.80 mmol) in a mixture of CH_2Cl_2–MeOH (1 : 1, 20 ml) was added at room temperature under stirring $Pb(OAc)_4$ (3.08 g, 6.95 mmol) within 2 min. After stirring for 15 min phosphate buffer (pH 7, 60 ml) was added and the solution was allowed to stir for further 45 min. The brown precipitate was filtered over Celite and the solid residue washed with CH_2Cl_2 (3 × 40 ml). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 40 ml). The organic phases were combined and the solvent was removed under reduced pressure. To the remaining yellow oil (1.5 g) was added hydrochloric acid (110 ml of a 6 N aq. solution) and the reaction mixture was refluxed for 9 h. The hot solution was decanted from the black residue and the solution was kept in a freezer at −30 °C overnight. The formed white precipitate was filtrated and washed with CH_2Cl_2 (2x 15 ml) and dried under high vacuum. The target compound (*S*)-**1** was obtained as a colourless powder (0.87 g, 64%). Mp: 300 °C (decomp.). ^{1}H NMR (300 MHz, TFA-d) δ 0.93 (s, 9 H), 1.88 (s, 6 H), 4.49 (s, 1 H); ^{13}C NMR (75 MHz, TFA-d) δ 26.6, 31.4, 36.8, 48.2, 50.5, 57.8, 174.5. Mass spectrum, *m*/*z* 198 (M^+–Cl, 100%). $[a]_D^{32}$ 38.7 (*c* 0.715 g per 100 ml in 0.1 N HCl). Found: C, 56.21; H, 8.84; N, 5.67, Cl, 14.87. $C_{11}H_{20}ClNO_2$ requires C, 56.52; H, 8.62; N, 5.99, Cl, 15.17%.

(*S*)-Fmoc-3-*tert*-Butylbicyclo[1.1.1]pent-1-yl)glycine (9)

To a suspension of amino acid hydrochloride (*S*)-**1** (1.00 g, 4.27 g) in a mixture of acetone–H_2O (1 : 1, 20 ml) was added Na_2CO_3 × 10 H_2O (0.65 g). In addition, sat. aqueous Na_2CO_3 solution was added until pH 9. When required, further Na_2CO_3 solution was added to maintain pH 9. To the white suspension was added *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (1.44 g, 4.27 mmol). After stirring for 12 h at room temperature the clear, yellow solution was diluted with ethyl acetate (20 ml) and carefully acidified with hydrochloric acid (3 N) to pH 2. The layers were separated and the aqueous phase extracted with ethyl acetate (2 × 20 ml). The combined organic layers were washed with hydrochloric acid (10 ml of a 3 N aq. solution), H_2O (5 ml) and dried over $MgSO_4$. The solvents were removed under reduced pressure to afford the protected amino acid **8** (1.66 g, 93%). Mp: 194–195 °C (CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 0.81 (s, 9 H), 1.51 (q, $J = 9.4$ Hz, 6 H), 4.08 (d, $J = 8.3$ Hz, 1 H), 4.16–4.34 (m, 3 H), 7.33 (t, $J = 7.3$ Hz, 2 H), 7.42 (t, $J = 7.0$ Hz, 2 H), 7.60 (d, $J = 8.3$ Hz, 1 H), 7.76 (d, $J = 7.5$ Hz, 2 H), 7.82 (d, $J = 7.2$ Hz, 2 H), 12.52 (br s, 1 H); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 25.8, 29.2, 35.6, 45.4, 46.7, 46.8, 55.3, 65.8, 120.1, 125.4, 127.1, 127.7, 140.7, 143.9, 156.2, 171.8. Found: C, 74.22; H, 6.76; N, 3.15. $C_{26}H_{29}NO_4$ requires C, 74.44; H, 6.97; N, 3.34%.

Antibacterial studies

E. coli (Gram-negative, DH 5α strain) and *B. subtilis* (Gram-positive, PY 22 strain) were used to test the antibacterial activity of the peptides.[7] Bacteria were cultivated to the mid-logarithmic phase of growth ($OD_{600} = 0.5$). Aliquots of the cell suspensions were added to the wells of a microtiter plate containing peptide at different concentrations. Final concentration of bacteria was 10^5 CFU per ml. Final concentrations of peptide ranged from 0.05 to 100 μM in 2-fold dilutions. Microtiter plates were incubated overnight at 37 °C and the absorbance was read at 600 nm (Autoreader EL 311, Bio-Tek Instruments Inc., USA). The minimum inhibitory concentration (MIC) of bacterial growth is defined as the lowest concentration of peptide at which there was no change in optical density.

Peptide-induced dye release from liposomes

To characterise the bilayer permeabilising activity against a lipid matrix mimicking the lipid composition of a bacterial membrane, peptide-induced calcein release from vesicles was determined fluorimetrically, as described.[7] Vesicles composed of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) and 1-palmitoyl-2-oleoylphosphatidyl-*sn*-glycerol (POPG) at a molar ratio of 3 : 1 were prepared by vortexing the dried lipid in dye buffer solution (70 mM calcein, 10 mM Tris, 0.1 mM EDTA, pH 7.4) and extrusion (Lipex Biomembranes Inc., Canada) through polycarbonate filters (six times through two stacked 0.4 μm pore size filters followed by eight times through two stacked 0.1 μm pore size filters). The fluorescence was excited at 490 nm and registered at 520 nm after 5 min after adding an aliquot of an LUV suspension to the peptide on a LS 50B spectrofluorimeter (Perkin Elmer Corp. Germany). The peptide concentration was 2 μM, the lipid concentration was 25 μM. The fluorescence intensity (*F*) corresponding to 100% dye release was determined by addition of bilayer-disturbing Triton X-100.

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Abbreviations

DMF, *N*,*N*-dimethylformamide; Fmoc, 9-fluorenylmethyloxycarbonyl; HAPyU, 1-(1-pyrrolidinyl-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene)pyrrolidinium hexafluorophosphate *N*-oxide; MALDI-MS, matrix-assisted laser desorption ionisation-mass spectrometry; MIC, minimal inhibitory concentration; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPG, 1-palmitoyl-2-oleoylphosphatidyl-*sn*-glycerol; RP-HPLC, reversed-phase high performance liquid chromatography; SPPS, solid phase peptide synthesis; TFA, trifluoroacetic acid; TMS-CN, trimethylsilylcyanide.

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