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Synthesis and biological evaluation of asymmetric gramicidin S analogues containing modified D-phenylalanine residues

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ABSTRACT

The synthesis of new analogues of the cationic antimicrobial peptide gramicidin S, having a modified D-phenylalanine residue, their antibacterial properties against several Gram positive and negative strains, as well as their hemolytic activity is reported.

1. Introduction

Prokaryotic and eukaryotic organisms employ antimicrobial peptides as part of their defense system against foreign invaders.[1] Some of these antimicrobial peptides are cationic in nature and are able to kill both Gram positive and Gram negative bacteria. This property identifies the cationic antimicrobial peptides as an attractive class of potential lead compounds for the development of novel antibiotics.[2] A major drawback of most cationic antimicrobial peptides however, is their toxicity toward mammalian cells.

Gramicidin S (GS, *cyclo*(Pro-Val-Orn-Leu-D-Phe)$_2$, Scheme 1), isolated from *Bacillus brevis*,[3] belongs to the class of cationic antimicrobial peptides. GS adopts a cyclic β-hairpin structure that is characterised by four intra-molecular hydrogen bonds and two two-residue turns formed by the D-phenylalanine-proline sequences.[4] The hydrophobic leucine and valine side-chains are displayed on one side of the molecule and the cationic side-chains of the ornithine residues on the other, leading to an overall amphiphilic structure. The amphiphilic nature of GS is thought to be at the basis of its antibacterial and hemolytic activities. Several GS derivatives have been designed and synthesised with the aim to find compounds with an improved biological profile.[5] Previous studies revealed that replacing D-phenylalanine with aromatic amino acid residues, such as D-pyrenylalanine, *para*-bromo-D-phenylalanine, D-serine(OBn) and dehydrophenylalanine, is well tolerated with respect to the antimicrobial activity.[6] Most of these reported GS derivatives with altered aromatic amino acid residues are symmetric in the sense that both D-phenylalanine residues are substituted. Often the effect of these modifications on the antimicrobial activity is not correlated with their cytotoxicity, as hemolytic data are not reported. We and others described the synthesis of GS analogues that are characterised by substitution of a single D-phenylalanine residue.[7] For instance, a GS derivative having one D-phenylalanine residue replaced by a D-tyrosine residue displayed diminished antibacterial activity. GS (D-tyrosine(OBn)) however, displayed the same activity as the natural product.[7f] In order to extend the scope of the modifications at this position of GS with respect to both antimicrobial activity and toxicity (hemolysis) we here present the synthesis of a series of asymmetric derivatives **1a–m** and their screening against several bacteria and red blood cells (Scheme 1).

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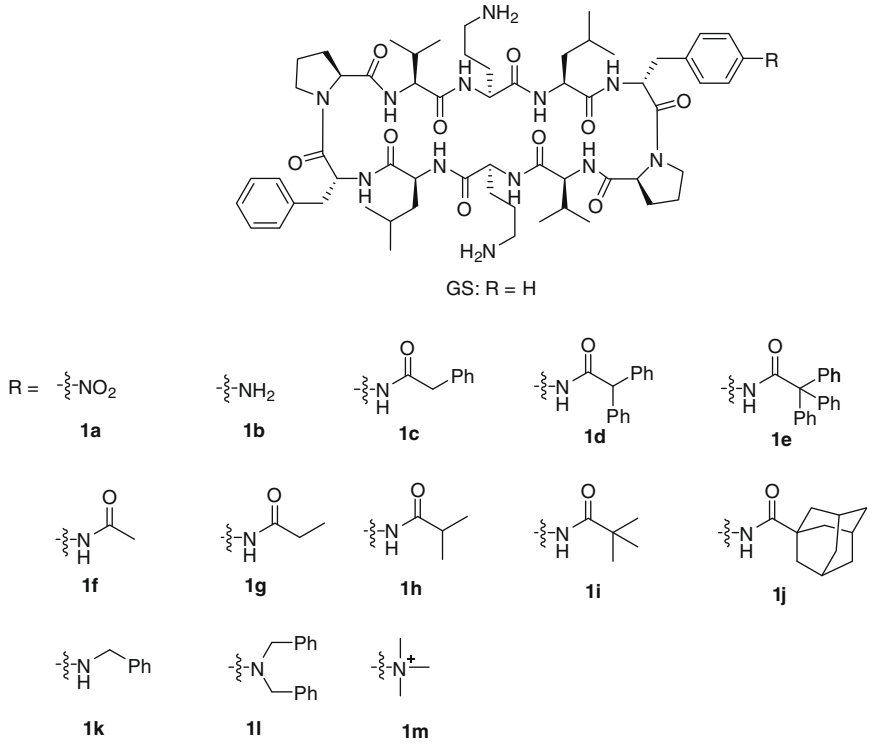
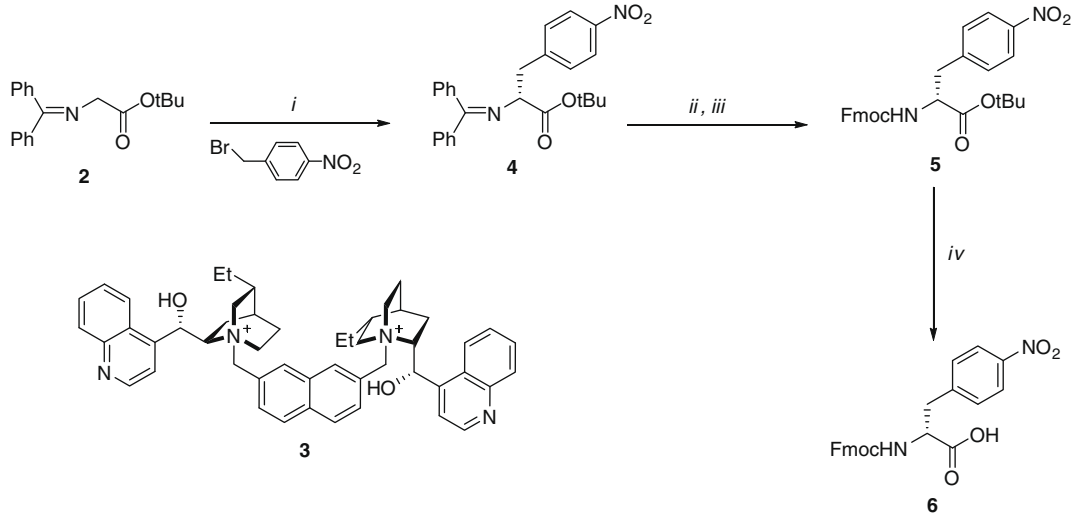


Scheme 1.

2. Results and discussion

Fmoc-protected *p*-nitro-D-phenylalanine **6**, key intermediate *en route* to GS derivatives **1a–m**, was synthesised starting from the commercially available glycine template **2** as outlined in Scheme 2. Imine ester **2** was enantioselectively alkylated with *p*-nitrobenzyl bromide under the agency of 5% of the chiral phase-transfer catalyst **3**, according to the literature procedure.[8] Product **4** was obtained in 84% yield and in 94% enantiomeric excess according to HPLC analysis using a column with a chiral stationary phase. After mild acidic hydrolysis of the benzophenone imine in **4**, the 9-fluorenylmethyleneoxycarbonyl (Fmoc) protecting group was introduced under Schotten–Baumann conditions to yield **5** in 76% yield over two steps. Finally, the *tert*-butyl ester was quantitatively removed by acidolysis with TFA to yield amino acid **6**.[9]

Nitro-gramicidin S derivative **9** was synthesised as follows (Scheme 3).[10] Fmoc-leucine derivatised HMPB-MBHA-resin[11] **7** was treated with 20% piperidine in NMP to remove the Fmoc group and subsequently condensed with N^{α}-Fmoc-N^{δ}-Boc-ornithine using HCTU as coupling reagent in the presence of DiPEA. This two-step cycle encompassing removal of the Fmoc group and coupling of the next amino acid was repeated until the fully assembled immobilised linear decapeptide having the *p*-nitro-D-phenylalanine residue at the *N*-terminus (**8**) was obtained. After removal

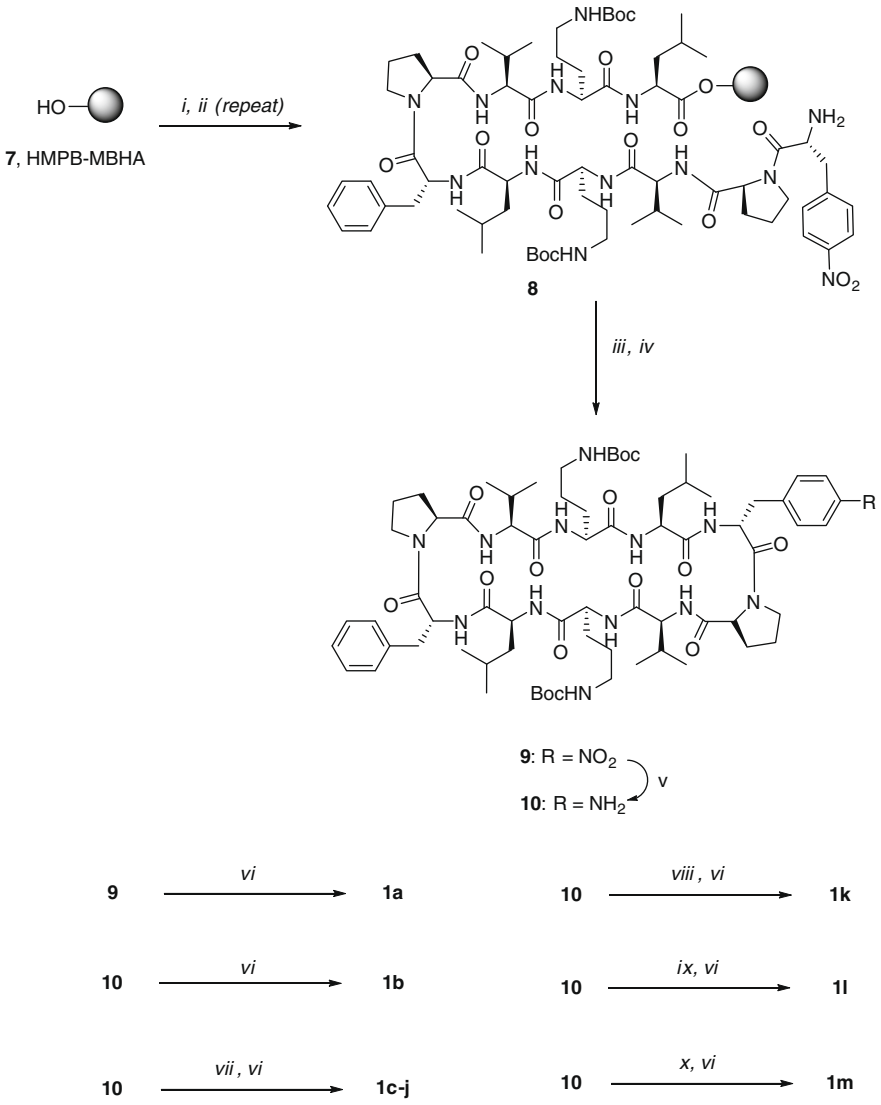


Scheme 2. Reagents and conditions: (i) 5 mol% **3** 50% aq KOH, $CHCl_3$/PhMe 84%, 94% ee; (ii) 15% citric acid, H_2O/THF; (iii) FmocOSu, K_2CO_3, H_2O/THF, 76% (two steps); (iv) 50% TFA, DCM, quant.

of the Fmoc group, the product was released from the solid support by treatment with 1% TFA in DCM, leaving the δ-amine Boc protected ornithine side chains intact. The obtained crude linear peptide was cyclised with PyBOP, HOBt and DiPEA under highly dilute conditions to obtain cyclic peptide **9**, which was purified by LH-20 gel-filtration. Peptide **9** was prepared on a gram scale in 87% overall yield. Reduction of the nitro group using $SnCl_2$ was accompanied by partial removal of the Boc-protecting groups. In an alternative procedure, the nitro functionality was selectively reduced by transfer hydrogenation using ammonium formate and 10% palladium on charcoal in ethanol, to produce aniline derivative **10** in quantitative yield.

Treatment of compound **9** with 50% TFA in DCM and subsequent HPLC purification gave compound **1a** in 30% yield. Via a similar deprotection–purification sequence compound **10** was transformed into **1b** (40% yield). The aromatic amine in compound **10** was acylated using the appropriate acid chloride in DCM with triethylamine as base. Subsequent removal of the Boc groups provided compound **1c–j** in yields ranging from 18% to 43%. Monobenzylation of **10** was affected by first forming the benzimine (benzaldehyde and triethylamine) and subsequent reduction with sodium borohydride. Acidolysis of the protective groups gave compound **1k** in 15% yield after HPLC purification. Double reductive amination of **10** with benzaldehyde and $NaBH_4$, however, did not provide the desired dibenzylated product **1l**. Compound **1l** was therefore prepared by benzylation of compound **10** using benzyl bromide, TBAI and sodium carbonate, which after Boc removal gave **1l** in 21% yield. The trimethylammonium salt **1m** was obtained using **10**, methyl iodide and sodium carbonate in DMF, and subsequent TFA treatment.

All GS derivatives **1a–m** were analysed by ^1H and ^{13}C NMR, and unambiguous signal assignments were made based on COSY and TOCSY spectra. The D-phenylalanine amide protons showed small J couplings (<4 Hz), indicating the presence of a β-turn, the $J_{NH,\alpha H}$ of the other NHs are all 8–9 Hz indicating an extended conformation, as expected.[12] In the ROESY spectra of compounds **1a–m** typical long-range and sequential ROE's were observed as indication of the cyclic β-hairpin structure. For instance, in the NH–NH interaction region of compound **1i**, a typical long range connectivity is present between the NHs of the valine and leucine residues (Fig. 1).



Scheme 3. Reagents and conditions: (i) deprotection: 20% piperdine/NMP; (ii) coupling: Fmoc-AA-OH, HCTU, DiPEA, NMP; (iii) 1% TFA, DCM; (iv) PyBOP, HOBt, DiPEA, DMF, 87% (starting from **7**; (v) HCO_2NH_4, 10% Pd/C, EtOH, quant.; (vi) 50% TFA/DCM, 30% (**1a**), 40% (**1b**), 38% (**1c**, two steps), 34% (**1d**, two steps), 38% (**1e**, two steps), 25% (**1f**, two steps), 18% (**1g**, two steps), 33% (**1h**, two steps), 27% (**1i**, two steps), 36% (**1j**, two steps), 15% (**1k**, two steps), 21% (**1l**, two steps), 30% (**1m**, two steps); (vii) RC(O)Cl, DiPEA, DCM; (viii) (a) $PhCH_2CHO$, Et_3N, MeOH; (b) $NaBH_4$, MeOH; (ix) BnBr, TBAI, Na_2CO_3, DMF; (x) MeI, Na_2CO_3, DMF.

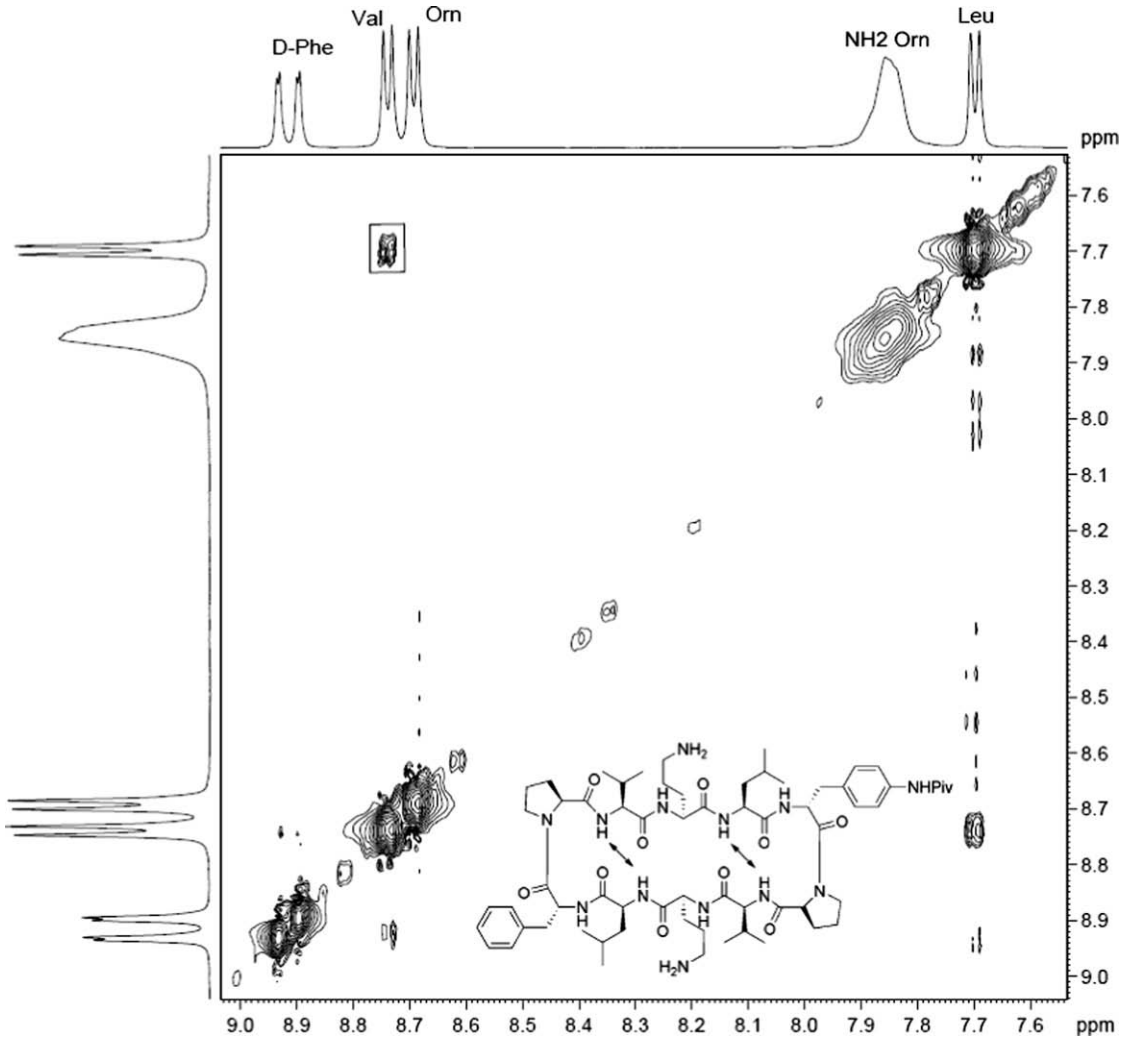


Figure 1. Part of the ROESY spectrum of compound **1i**. (CD_3OH, 600 MHz, mixing time 300 ms) Indicated is the observed ROE coupling and its assignment.

Two of the GS derivatives (**1c** and **1g**) furnished crystals suitable for X-ray analysis. The structures of **1c** and **1g** were both solved and refined to 1.1 Å resolution. Figure 2 shows the overlaid side- and top-views of the different conformers of **1c** and **1g** as found in their unit cells. The X-ray structures of both derivatives compare well with the cyclic β-hairpin structure of gramicidin S.[4f] The β-turn motifs and the amphiphilic nature are retained, though the modified D-phenylalanine side chains exhibit disorder and can adopt different conformations in the crystal lattice and, presumably, in solution. In the crystals, multiple molecules of both **1c** and **1g** adopt helical channels, with the hydrophilic ornithine residues facing the centre and the hydrophobic valine and leucine residues on the exterior (not shown). The refined X-ray structures compare well with the information obtained from the NMR analyses.

The antimicrobial activity of peptides **1a–m** was established by determination of the minimal inhibitory concentration (MIC) in a standard screening assay in broth on several Gram-positive and Gram-negative bacterial strains (Table 1). For comparison, the antibacterial activity of GS is included in Table 1. *Staphylococcus aureus*, *CNS*, *Enterococcus faecalis* and *Steptococcus mitis* are Gram-positive species, whereas *Escherichia coli* and *Pseudomonas aeruginosa* are Gram-negative species. The MIC values obtained for GS are comparable with our previously reported data.[13] Within the series **1c**, **1d**, **1e**, the antimicrobial activity decreases with increasing bulk and hydrophobicity of the turn region. Within the series **1g**, **1h**, **1i**, the antimicrobial activity increases with increasing bulk and hydrophobicity of the turn region. Compound **1j**, yet more hydrophobic and bulkier than **1i**, shows similar activity as GS for the Gram-positive bacteria, but is less active against Gram-negative bacteria. The other derivatives are substantially less active than GS, indicating that the presence of an amine functionality is not favorable with respect to biological activity.

The toxicity of compounds **1a–m** towards human erythrocytes was examined by a standard twofold dilution assay of the peptides as compared with a blank measurement and 100% hemolysis induced by 1% Triton X-100 in saline. The hemolytic activity of GS agrees with the literature (EC_{50} = 11.7 μM).[13] The found EC_{50} values ranged from 9.35 μM for **1c** to 134 μM for **1m**. Compounds **1c** and **1j** display hemolytic activity comparable with GS. Compound **1i** is slightly less hemolytic: 50% hemolysis at 25 μM, compared to 12 μM for GS. The compounds **1f** and **1m** are the least hemolytic of the series (Fig. 3).

3. Conclusion

Previously, we synthesised several derivatives of GS having modifications at the turn residues proline and D-phenylalanine.[7f] In our hands, modifications at proline were not well tolerated with respect to retaining antibacterial activity, with the exception of the

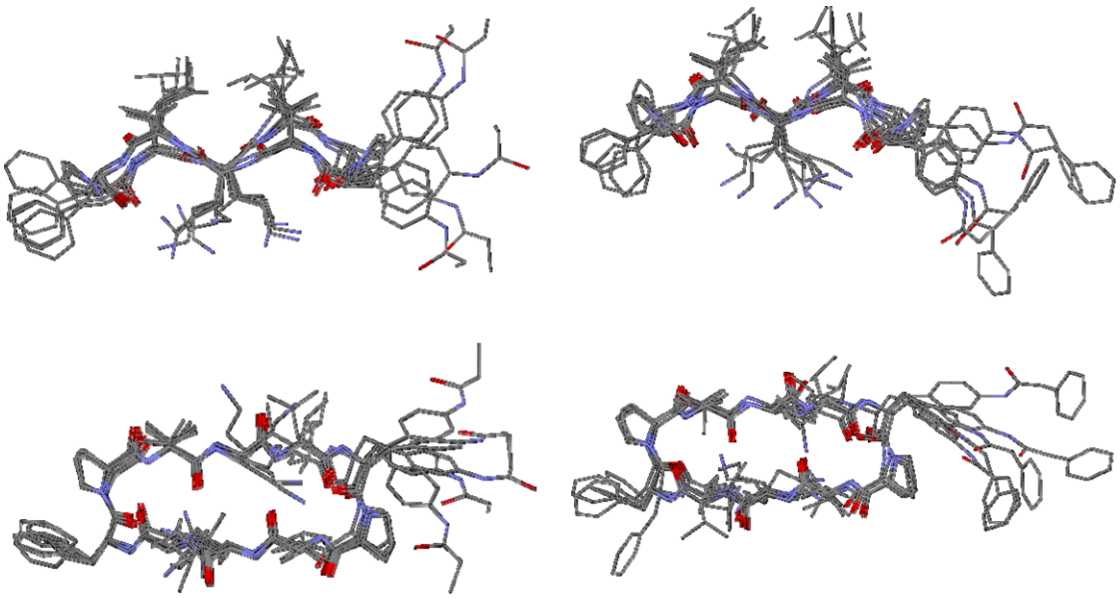


Figure 2. Side- and top views of compounds **1c** (left top and bottom panels) and **1g** (right top and bottom panels). The peptide backbones of the five molecules in the crystallographic asymmetric units are overlaid.

Table 1
Antimicrobial screening of compounds **1a–m** and GS in several Gram-positive/negative bacterial strains (MIC in μg/mL)

Strain	GS	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k	1l	1m
Staphylococcus aureus 7323	8	16	32	8	16	>64	64	32	32	8	8	>64	>64	64
Staphylococcus aureus 7388	8	16	16	16	32	>64	16	16	16	8	8	64	>64	32
CNS 5277	16	16	16	8	64	>64	16	8	16	8	16	64	64	16
CNS 5115	16	16	32	8	>64	>64	32	16	16	8	8	>64	>64	16
CNS 7368	8	16	16	8	8	>64	32	8	16	8	8	64	64	32
Enterococcus faecalis 1131	16	32	64	32	16	>64	64	32	16	16	64	>64	>64	>64
Escherichia coli ATCC25922	32	64	64	32	>64	>64	64	64	32	32	>64	>64	>64	64
Pseudomonas aeruginosa AK1	64	>64	>64	>64	>64	>64	>64	>64	>64	64	>64	>64	>64	>64
Pseudomonas aeruginosa ATCC19582	8	>64	>64	>64	>64	>64	>64	>64	>64	64	>64	>64	>64	>64
Streptococcus mitis BMS	8	32	8	8	16	64	8	8	8	4	8	64	64	8
Streptococcus mitis ATCC33399	4	32	32	16	>64	>64	16	16	32	4	16	64	64	8

azide functionality, while modification of D-phenylalanine appeared promising.[7f] To elaborate on this observation we synthesised a series of GS derivatives with modifications at D-phenylalanine. Incorporation of modified D-phenylalanine residues does not influence the structure in solution, as gauged by extensive NMR experiments, and compounds **1a–m** all adopt cyclic β-hairpin secondary structures. The NMR data is in agreement with the X-ray structures as obtained for the derivatives **1c** and **1g**.

The general trend observed for the here studied series of *para*-substituted D-phenylalanine derivatives of GS is that less hemolytic derivatives are also less antimicrobial. Compounds **1f** and **1m** are the least hemolytic in these series, however still have antimicrobial activity against *Streptococcuc mitis* BMS. Compounds **1k** and **1l** do not possess any antimicrobial activity, indicating that the presence of an amine functionality is not favourable with respect to biological activity.

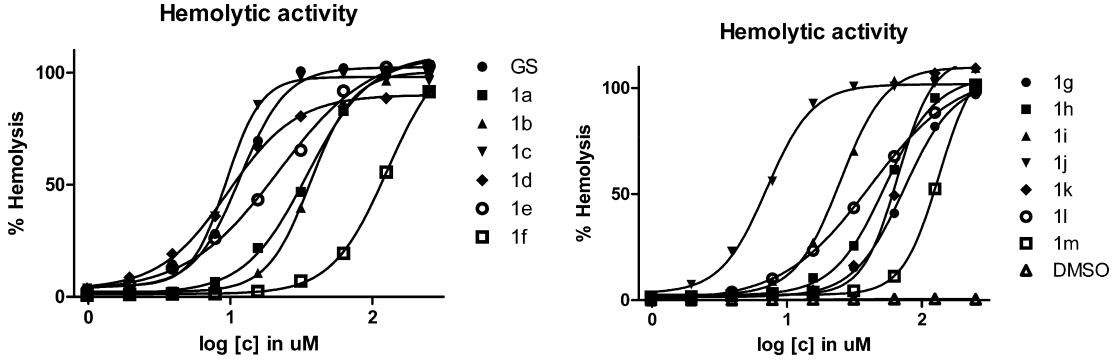


Figure 3. Hemolytic activity of compounds **1a–f** and GS (left panel) and **1g–m** (right panel).

When comparing the series **1c**, **1d**, **1e**, and **1g**, **1h**, **1i**, it appears that there is an optimum in size and hydrophobicity of the turn region with respect to retaining the antimicrobial activity. Compound **1j**, being bulkier and more hydrophobic than **1i** but less bulky and hydrophobic than **1e** is selective for Gram-positive bacteria. The most promising compound of the here described series of compounds is derivative **1i** that combines a comparable antimicrobial activity as GS with a slightly reduced hemolytic activity.

4. Experimental

Solvents and chemicals were used as received from their supplier. Solvents were stored over 4 Å molecular sieves (or 3 Å MS for MeOH). Solvents for extractions and silica gel chromatography were of technical grade and distilled before use. Chemicals were used as received from their suppliers, without any further purification. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV-400 (400/100 MHz), or Bruker DMX-600 spectrometer (600/150 MHz). Chemical shifts δ are given in ppm relative to tetramethylsilane (0 ppm) or MeOD (3.31 ppm) as internal standard. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in water/acetonitrile; 50:50 v/v and 0.1% formic acid) on a mass spectrometer (Termo Finnigan LTQ Orbitrap) equipped with an electro spray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60,000 at m/z 400 (mass range m/z = 150–2000) and dioctylphthalate (m/z = 391.28428) as a 'lock mass'.[14] The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). LC/MS analyses were performed on a LCQ Adventage Max (Thermo Finnigan) equipped with a Gemini C18 column (Phenomenex). The applied buffers were A: H_2O, B: MeCN and C 1.0% aq TFA. Compound **4** was synthesised in both racemic and non-racemic form. Enantiomeric excesses were determined by comparing racemic with non-racemic compounds by chiral HPLC employing a Daicel CHIRALCEL OD column, using hexane 2-propanol mixtures as the eluent (1.0 mL min^{-1}), and UV detection at 254 nm.

4.1. Antimicrobial screening

Bacteria were stored at −70 °C and grown at 30 °C on Columbia Agar with sheep blood (Oxoid, Wesel, Germany) suspended in physiological saline until an optical density of 0.1 AU (at 595 nm, 1 cm cuvette). The suspension was diluted (10×) with physiological saline, and 2 μL of this inoculum was added to 100 μL growth medium, Nutrient Broth from Difco (ref. nr. 234000, lot nr. 6194895) with yeast extract (Oxoid LP 0021, lot nr. 900711, 2 g/400 mL broth) in Microtiter plates (96 wells). The peptides GS and **1a–m** were dissolved in ethanol (4 g/L) and diluted in distilled water (1000 mg/L), and twofold diluted in the broth (64, 32, 16, 8, 4 and 1 mg/L). Incubation at 30 °C (24–96 h) and the MIC was determined as the lowest concentration inhibiting bacterial growth (Table 2).

4.2. Hemolytic assay

Freshly drawn heparinised blood was centrifuged for 10 min at 1000*g* at 10 °C, Subsequently, the erythrocyte pellet was washed three times with 0.85% saline solution and diluted with saline to a 1/25 packed volume of red blood cells. The peptides to be evaluated were dissolved in a 30% DMSO/0.5 mM saline solution to give a 1.5 mM solution of peptide. If a suspension was formed, the suspension was sonicated for a few seconds. A 1% Triton-X solution was prepared. Subsequently, 100 μL of saline solution was dispensed in columns 1–11 of a microtiter plate, and 100 μL of 1% Triton solution was dispensed in column 12. To wells A1–C1, 100 μL of the peptide was added and mixed properly. 100 μL of wells A1–C1 was dispensed into wells A2–C2. This process was repeated until wells A10–C10, followed by discarding 100 μL of wells A10–C10. These steps were repeated for the other peptides. Subsequently, 50 μL of the red blood cell solution was added to the wells and the plates were incubated at 37 °C for 4 h. After incubation, the plates were centrifuged at 1000*g* at 10 °C for 4 min. In a new microtiter plate, 50 μL of the supernatant of each well was dispensed into a corresponding well. The absorbance at 405 nm was measured and the percentage of hemolysis was determined.

4.3. Chemistry

4.3.1. Stepwise elongation

Fmoc-Leu-HMPB-MBHA resin (Loading of the resin was 0.56 mmol/g, 2.8 mmol) was submitted to nine cycles of Fmoc solid-phase synthesis with the appropriate commercial amino acid building blocks, or Fmoc-*p*-nitro-D-Phe-OH. The amino group on the side-chain of ornithine was protected with a Boc-group. Fmoc removal was effected by treatment with 20% piperidine in NMP for 2 × 10 min. The resin was subsequently washed with NMP, DCM, MeOH, and finally NMP. The Fmoc-AA-OH (7 mmol, 2.5 equiv), HCTU (7 mmol, 2.5 equiv) in NMP was pre-activated for 1 min after the addition of DiPEA (11.2 mmol, 3 equiv) and then added to the resin. The suspension was shaken for 1.5 h. The resin was washed with NMP, DCM, MeOH and NMP.

4.3.2. Cleavage from the resin

After the final Fmoc deprotection the resin was washed with NMP and DCM and treated with 50 mL 1% TFA in DCM (6 × 10 min). The filtrates were collected and coevaporated with toluene (3 × 100 mL).

4.3.3. Cyclisation

In DMF (1L) were dissolved PyBOP (7 mmol, 5 equiv), HOBt (7 mmol, 5 equiv), and DiPEA (21 mmol, 15 equiv). The linear decapeptide (1.4 mmol) was dissolved in DMF (40 mL) and added dropwise over 1 h to the coupling cocktail. After addition the mixture was stirred for 16 h. The reaction mixture was concentrated in vacuo and the crude mixture was subjected to LH-20 size exclusion chromatography to yield cyclic peptide **9** in 87% yield (1.45 g) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$; rotamers) δ 8.74–8.59 (m), 8.15 (br s), 7.60–7.22 (m), 6.77 (br s), 6.42 (br s), 5.01–4.42 (m), 3.84–3.62 (m), 3.15 (br s), 2.06 (br s), 1.89–1.61 (m), 1.54–1.39 (m), 0.89 (s); ^{13}C NMR (100 MHz, $CDCl_3$; rotamers) δ 172.01, 171.89, 171.34, 170.87, 170.54, 170.48, 170.26, 170.05, 169.83, 169.41, 156.01, 155.92, 146.87, 143.83, 135.75, 130.22, 129.22, 128.30, 127.08, 123.47, 78.06, 77.96, 77.20, 60.26, 60.00, 53.43, 41.81, 40.60, 40.49, 40.46, 28.30, 24.54, 24.34, 22.95, 22.73, 18.94, 18.68, 18.59, 18.31, 17.18, 11.68; HRMS: calcd for $[C_{70}H_{107}N_{13}O_{16}+H]^+$: 1386.80315, found: 1386.80447. RP-HPLC: 70–90%, R_t = 4.91 min.

4.3.4. Turn modifications

Compound **1a**: compound **9** (40 mg, 29 μmol) was dissolved in DCM (2 mL) and TFA (2 mL) was added. When LC/MS indicated complete Boc removal, the reaction mixture was concentrated under reduced pressure. The residue was purified using HPLC using acetonitrile and water containing 1% trifluoroacetic acid as eluent, yielding compound **1a** (10 mg, 9 μmol, 30%) as a colorless oil.

^{13}C NMR (151 MHz, MeOH) δ 173.77, 173.08, 173.02, 172.52, 148.80, 144.87, 136.96, 131.73, 130.50, 129.80, 128.62, 124.75, 86.79, 62.24, 62.09, 60.53, 56.08, 55.25, 52.59, 51.51, 48.34, 48.04, 42.10, 42.00, 40.70, 37.39, 36.82, 32.12, 32.07, 30.87, 30.75, 25.74, 24.76, 24.74, 24.55, 23.31, 23.14, 23.10, 19.75,

Table 2
Proton NMR signals (600 MHz, CD_3OH). For amide signals the $J_{NH,\alpha H}$ are given in parantheses.

Peptide	Residue	NH amide	H_α	H_β	H_γ	H_δ	NH_2
1a	^{D}Phe	8.97 (3.21)	4.62	3.20/3.10	8.21–7.23		
		8.94 (3.11)	4.49	3.09/2.94			
	Val	8.75 (9.11)	4.66	1.52/1.39	0.89		
		8.74 (9.48)					
	Orn	8.71 (8.72)	4.97	1.60	1.76	3.02/2.87	7.84
	Leu	7.71 (9.01)	4.15	2.27	0.97	0.89	
	Pro		4.38	1.73	2.03	3.87/2.70	
			4.32	1.67	1.99	3.72/2.46	
1b	DPhe	8.94 (3.03)	4.53	3.08/2.94	7.39–7.11		
		8.92 (3.23)					
	Val	8.74 (9.37)	4.66	1.54/1.39	0.90		
	Orn	8.71 (8.74)	4.97	1.66	1.77	2.03	7.85
	Leu	7.71 (8.74)	4.16	2.29	0.98	0.88	
		7.69 (8.71)					
	Pro		4.39	1.75	2.04	3.80/2.60	
			4.34	1.70	2.01	3.73/2.47	
1c	R	10.04	3.66				
	DPhe	8.93 (3.07)	4.49	3.08/2.93	7.49–7.19		
		8.89 (3.05)	4.47	3.05/2.89			
	Val	8.73 (9.31)	4.65	1.52/1.39	0.88		
		8.72 (9.36)					
	Orn	8.69 (9.31)	4.97	2.03	1.74/1.59	3.03/2.86	7.87
	Leu	7.70 (8.97)	4.15	2.29	0.95	0.87	
		7.69 (8.98)					
	Pro		4.36	1.72	2.02	3.72	
1d	R	10.22	1.93				
	DPhe	8.93 (3.04)	4.48	3.07/2.92	7.51–7.19		
		8.90 (2.99)					
	Val	8.73 (9.29)	4.64	1.50/1.37	0.87		
		8.73 (9.35)					
	Orn	8.70 (9.33)	4.95	2.03	1.74/1.57	3.03/2.85	7.83
	Leu	7.70 (8.87)	4.12	2.25	0.95	0.86	
		7.69 (8.84)					
	Pro		4.33	2.00/1.68	1.77/2.49	3.72/2.55	
1e	DPhe	8.93 (3.00)	4.49	3.06/2.91	7.36–7.17		
		8.90 (2.97)	4.44	3.04/2.87			
	Val	8.73 (10.03)	4.64	1.51/1.37	0.87		
		7.72 (10.66)					
	Orn	8.69 (9.41)	4.94	2.00	1.71/1.57	3.13	7.83
	Leu	7.70 (9.13)	4.12	2.24	0.93	0.86	
		7.68 (9.14)					
	Pro		4.33	1.98/1.66	2.44	3.71	
			4.29	1.96/1.69	2.51	3.69	
1f	R	9.89	2.11				
	DPhe	8.93 (3.11)	4.51	3.10/2.93	7.48–7.18		
		8.90 (3.04)	4.51	3.09/2.91			
	Val	8.74 (9.67)	4.67	1.52/1.39	0.89		
		8.73 (9.67)					
	Orn	8.70 (9.39)	5.00	2.05	1.75/1.62	3.03/2.86	7.84
	Leu	7.71 (9.00)	4.16	2.27	0.95	0.87	
	Pro		4.35	2.00/1.74	2.56/2.47	3.75	
1g	R	9.76	2.38	1.18			
	DPhe	8.93 (3.15)	4.5	3.09/2.93	7.50–7.18		
		8.90 (3.12)	4.48	3.04/2.91			
	Val	8.73 (9.35)	4.66	1.52/1.40	0.89		
	Orn	8.69 (9.25)	4.97	2.04	1.76/1.60	3.03/2.88	7.86
	Leu	7.70 (8.67)	4.15	2.26	0.95	0.87	
	Pro		4.35	2.00/1.74	2.56/2.47	3.75	
1h	R	9.73	2.60	1.18			
	DPhe	8.93 (3.10)	4.50	3.09/2.93	7.50–7.19		
		8.89 (3.06)	4.49	3.05/2.90			
	Val	8.73 (9.41)	4.66	1.53/1.39	0.89		
	Orn	8.70 (9.22)	4.98	2.04	1.74/1.59	3.03/2.86	7.84
	Leu	7.71 (8.76)	4.15	2.26	0.95	0.87	
	Pro		4.35	1.65/1.57	2.58/2.47	3.77/3.73	
1i	R	9.13		1.28			
	DPhe	8.93 (d, 3.2)	4.50	3.08/2.93	7.46–7.19		
		8.90 (d, 3.2)	4.50	3.05/2.93			
	Val	8.74 (d, 9.3)	4.66	1.52/1.39	0.88		
	Orn	8.69 (d, 9.2)	4.97	2.03	1.75/1.62	3.02/2.87	7.86
	Leu	7.70 (d, 8.9)	4.14	2.26	0.95	0.87	
	Pro		4.35	1.77/1.68	2.60/2.46	3.77/3.73	

Table 2 (*continued*)

Peptide	Residue	NH amide	H_α	H_β	H_γ	H_δ	NH_2
1j	R	9.03		2.07–1.47			
	DPhe	8.93 (3.08)	4.5	3.09/2.94	7.70–7.17		
		8.89 (3.07)	4.5	3.06/2.91			
	Val	8.73 (9.37)	4.67	1.53/1.40	0.89		
	Orn	8.71 (9.34)	4.98	2.04	1.76/1.60	3.03/2.86	7.50
	Leu	7.71 (8.97)	4.15	2.27	0.95	0.88	
	Pro		4.35	2.00/1.79	2.61	3.78	
			4.35	2.00/1.68	2.47	3.74	
1k	DPhe	8.95 (br s)	4.49	3.09/2.95	7.47–6.70		
	Val	8.74 (9.23)	4.65	1.53/1.39	0.89		
		8.73 (9.29)					
	Orn	8.70 (9.79)	4.96	2.10/1.61	1.77	3.00/2.86	7.87
		8.66 (9.36)					
	Leu	7.68 (8.86)	4.14	2.26	0.95/0.88		
	Pro		4.34	1.69	2.46/1.99	3.73	
			4.28	1.55	2.46/1.94	3.67	
1l	DPhe	8.93 (3.24)	4.59	3.20/3.06	6.64–7.32		
		8.88 (3.17)	4.45	3.04/2.87			
	Val	8.73 (9.34)	4.74	1.64/1.50	0.99		
		8.70 (9.35)					
	Orn	8.66 (9.20)	5.05	2.11/1.71	1.86	3.10/2.96	7.96
	Leu	7.68 (8.88)	4.24	2.38	1.05/0.98		
		7.67 (8.95)					
	Pro		4.19	1.91/1.46	1.65	2.34/2.47	
1m	R		3.66				
	DPhe	8.94 (br s)	4.58	3.16/3.08	7.87–7.23		
	Val	8.75 (8.68)	4.66	1.51/1.40	0.90		
		8.74 (8.73)					
	Orn	8.71 (9.00)	4.97	2.03/1.60	1.76	3.04/2.89	7.87
		8.70 (8.94)					
	Leu	7.70 (9.00)	4.15	2.26	0.94	0.87	
		7.65 (8.97)	4.17	2.26	0.96	0.88	
	Pro		4.42	2.07/1.79	2.72	3.88	
			4.35	2.00/1.68	2.46	3.73	

19.57; HRMS: calcd for $[C_{60}H_{91}N_{13}O_{12}+H]^{2+}$: 593.85278, found: 593.85265. RP-HPLC: 10–90%, R_t = 8.43 min.

Compound **1b**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** was dissolved in DCM (2 mL) and TFA (2 mL) was added. When LC/MS indicated complete Boc removal, the reaction mixture was concentrated under reduced pressure. The residue was purified using, yielding compound **1b** (13 mg, 14 μmol, 40%) as a colorless oil.

^{13}C NMR (151 MHz, MeOH) δ 173.68, 173.66, 173.64, 173.55, 173.54, 172.90, 172.87, 172.54, 172.52, 136.98, 131.89, 130.50, 129.79, 128.61, 121.57, 62.14, 62.09, 60.51, 56.07, 55.94, 52.57, 51.57, 51.55, 48.15, 48.03, 42.11, 40.70, 37.39, 36.66, 32.11, 32.07, 30.86, 30.79, 30.75, 25.76, 24.74, 24.62, 24.55, 23.30, 23.15, 19.76, 19.56; HRMS: calcd for $[C_{60}H_{93}N_{13}O_{10}+H]^{2+}$: 578.86569, found: 578.86551. RP-HPLC: 10–90%, R_t = 6.00 min.

Compound **1c**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (36 μmol) was coevaporated twice with dichloroethane and redissolved in DCM (2 mL). Under an argon atmosphere Et_3N (3 equiv, 15 μL μL) was added followed by phenylacetyl chloride (3 equiv, 14 μL). Stirring was continued for 30 min. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After stirring for 1 h, the reaction mixture was concentrating in vacuo and HPLC purification, provided compound **1c** in 38% yield (14 μmol, 17 mg) as a colorless oil.

^{13}C NMR (151 MHz, MeOH) δ 173.70, 173.66, 173.62, 173.56, 172.88, 172.56, 172.53, 172.52, 139.37, 136.98, 136.79, 132.72, 130.89, 130.57, 130.15, 129.79, 129.64, 128.60, 128.03, 121.71, 62.09, 60.51, 56.07, 55.99, 52.55, 51.56, 49.58, 48.11, 48.00, 44.72, 42.10, 40.69, 40.59, 37.40, 36.79, 32.07, 30.89, 30.74, 25.76, 24.72, 24.57, 23.30, 23.14, 19.75, 19.56; HRMS: calcd for $[C_{68}H_{99}N_{13}O_{11}+H]^{2+}$: 637.88663, found: 637.88690. RP-HPLC: 10–90%, R_t = 8.62 min.

Compound **1d**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (36 μmol) was coevaporated twice with dichloroethane and redissolved in DCM (2 mL) and put under an argon atmosphere. First Et_3N (3 equiv, 15 μL) was added, then diphenylacetyl chloride (3 equiv, 26 mg). After 60 min TLC ($CHCl_3$/PhMe 9:1) indicated completion and the reaction was concentrated. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After stirring for 1 h, concentrating in vacuo and HPLC purification, compound **1d** was obtained in 34% yield (12 μmol, 17 mg) as a colorless oil.

^{13}C NMR (151 MHz, MeOH) δ 173.69, 173.65, 173.61, 173.55, 173.17, 172.87, 172.52, 172.51, 140.99, 139.42, 136.98, 132.78, 130.93, 130.50, 129.89, 129.79, 129.54, 128.60, 128.21, 121.69, 62.13, 62.09, 60.50, 59.71, 56.06, 55.98, 52.55, 51.56, 48.11, 48.03, 42.09, 40.69, 37.39, 36.79, 32.08, 30.89, 30.76, 25.76,

24.72, 24.56, 23.30, 23.14, 19.75, 19.56; HRMS: calcd for $[C_{74}H_{103}N_{13}O_{11}+H]^{2+}$: 675.90228, found: 675.90263. RP-HPLC: 10–90%, R_t = 8.70 min.

Compound **1e**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (38 μmol) was coevaporated twice with dichloroethane and redissolved in DCM (2 mL). Under an argon atmosphere Et_3N (3 equiv, 16 μL) was added, followed by triphenylacetyl chloride[16] (3 equiv, 35 μL). Stirring was continued for 3 h and the reaction mixture was concentrated. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After stirring for 1 h, concentrating under reduced pressure and HPLC purification, compound **1e** was obtained in 38% yield (14 μmol, 18 mg) as a colorless oil.

^{13}C NMR (151 MHz, MeOH) δ 174.38, 173.69, 173.55, 172.85, 172.51, 144.61, 138.75, 136.98, 133.41, 131.65, 130.82, 130.50, 129.79, 129.06, 128.13, 123.08, 62.13, 60.50, 56.07, 55.98, 55.92, 52.54, 51.55, 47.99, 47.42, 42.09, 40.66, 32.08, 30.87, 30.70, 27.43, 25.75, 25.05, 24.72, 24.56, 23.31, 23.14, 19.75, 19.56, 9.26; HRMS: calcd for $[C_{80}H_{107}N_{13}O_{11}+H]^{2+}$: 713.91793, found: 713.91824. RP-HPLC: 10–90%, R_t = 9.77 min.

Compound **1f**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (38 μmol) was coevaporated twice with dichloroethane and redissolved in DCM (2 mL). Under an argon atmosphere Et_3N (3 equiv, 16 μL) was added, followed by acetyl chloride (3 equiv, 8 μL). Stirring was continued for 30 min and the reaction mixture concentrated under reduced pressure. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After stirring for 1 h, concentrating under reduced pressure and HPLC purification, compound **1f** was obtained in 25% yield (9 μmol, 11 mg) as an off-white oil.

^{13}C NMR (151 MHz, MeOH) δ 173.72, 173.68, 173.67, 173.67, 173.54, 172.89, 172.89, 172.49, 171.86, 139.41, 136.96, 132.53, 130.85, 130.85, 130.49, 129.79, 129.79, 128.81, 128.61, 121.58, 121.58, 62.14, 62.10, 60.51, 56.06, 56.00, 52.54, 51.55, 49.58, 49.43, 49.29, 49.15, 49.01, 48.87, 48.72, 48.12, 48.04, 42.10, 40.70, 37.39, 36.77, 32.09, 30.88, 30.78, 30.75, 25.76, 24.73, 24.58, 24.55, 23.84, 23.31, 23.14, 19.76, 19.57; HRMS: calcd for $[C_{62}H_{95}N_{13}O_{11}+H]^{2+}$: 599.87098, found: 599.87095. RP-HPLC: 10–90%, R_t = 7.82 min.

Compound **1g**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (36 μmol) was coevaporated twice with dichloroethane and redissolved in DCM (2 mL). Under an argon atmosphere, Et_3N (5 equiv, 25 μL) was added, followed by propionyl chloride (3 equiv, 10 μL). Stirring was continued for 2 h and the reaction concentrated. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After stirring for 1 h, concentrating under reduced pressure and HPLC purification, compound **1g** was obtained in 18% yield (6 μmol, 8 mg) as a colorless oil.

^{13}C NMR (151 MHz, MeOH) δ 175.57, 173.69, 173.65, 173.56, 172.87, 172.87, 172.53, 139.48, 136.99, 132.43, 130.85, 130.50, 129.78, 128.59, 121.57, 62.14, 62.09, 60.51, 56.07, 56.01, 52.56, 51.57, 48.12, 48.03, 42.10, 40.69, 37.40, 36.79, 32.07, 31.03, 30.90, 30.78, 30.73, 25.76, 24.73, 24.59, 24.55, 23.31, 23.15, 19.75, 19.56, 10.23; HRMS: calcd for $[C_{63}H_{97}N_{13}O_{11}+H]^{2+}$: 606.87880, found: 606.87884. RP-HPLC: 10–90%, R_t = 7.45 min.

Compound **1h**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (38 μmol) was coevaporated twice with dichloroethane and redissolved in DCM (2 mL). Under an argon atmosphere, Et_3N (3 equiv, 16 μL) was added, followed by isobutyryl chloride (3 equiv, 12 μL). After 30 min the reaction was concentrated. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After stirring for 1 h, concentrating under reduced pressure and HPLC purification, compound **1h** was obtained in 33% yield (12 μmol, 15 mg) as a colorless oil.

^{13}C NMR (151 MHz, MeOH) δ 178.81, 173.74, 173.67, 173.55, 172.90, 172.50, 139.53, 136.97, 132.46, 130.84, 130.49, 129.79, 128.81, 128.61, 121.71, 115.06, 115.01, 62.16, 62.10, 60.51, 56.07, 55.99, 52.54, 51.55, 49.58, 49.43, 49.29, 49.15, 49.01, 48.87, 48.72, 48.15, 48.04, 42.10, 40.70, 40.60, 37.40, 37.05, 36.78, 32.09, 30.88, 30.81, 30.76, 25.76, 24.73, 24.59, 24.55, 23.31, 23.14, 19.95, 19.91, 19.76, 19.56; HRMS: calcd for $[C_{64}H_{99}N_{13}O_{11}+H]^{2+}$: 613.88663, found: 613.88671. RP-HPLC: 10–90%, R_t = 8.42 min.

Compound **1i**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (36 μmol) was coevaporated twice with dichloroethane and redissolved in DCM (2 mL). Under an argon atmosphere, Et_3N (10 equiv, 50 μL) was added, followed by pivaloyl chloride (5 equiv, 22 μL). Stirring was continued for 8 h and the reaction was concentrated. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After stirring for 1 h, concentrating under reduced pressure and HPLC purification, compound **1i** was obtained in 27% yield (10 μmol, 12 mg) as a colorless oil.

^{13}C NMR (151 MHz, MeOH) δ 180.14, 173.73, 173.73, 173.64, 173.61, 173.56, 172.86, 172.86, 172.54, 172.54, 139.29, 136.98, 132.89, 130.69, 130.50, 129.78, 128.59, 123.16, 62.17, 62.08, 60.52, 56.07, 55.98, 52.56, 51.56, 48.15, 48.02, 42.09, 40.69, 40.59, 40.54, 37.39, 36.77, 32.06, 30.89, 30.79, 27.81, 25.75, 24.73, 24.60, 24.54, 23.31, 23.14, 19.75, 19.58; HRMS: calcd for $[C_{65}H_{101}N_{13}O_{11}+H]^{2+}$: 620.89445, found: 620.89479. RP-HPLC: 10–90%, R_t = 8.41 min.

Compound **1j**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (36 μmol) was coevaporated twice with dichloroethane and redissolved in DCM (2 mL). Under an argon atmosphere, Et_3N (3 equiv, 15 μL μL) was added, followed by acetyl chloride (3 equiv, 22 mg). After 4 h the reaction was concentrated. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After stirring

for 1 h, concentrating under reduced pressure and HPLC purification compound **1j** was obtained in 36% yield (13 μmol, 17 mg).

^{13}C NMR (151 MHz, MeOH) δ 173.76, 173.68, 173.67, 173.63, 173.56, 172.90, 172.89, 172.51, 139.23, 136.98, 132.88, 130.68, 130.49, 129.79, 128.81, 128.61, 123.20, 115.06, 115.01, 70.36, 62.18, 62.10, 60.53, 56.07, 55.97, 52.54, 51.55, 48.16, 48.03, 42.09, 40.70, 40.59, 40.07, 37.56, 37.40, 36.77, 32.07, 30.88, 30.81, 30.75, 29.74, 25.76, 24.73, 24.68, 24.61, 24.55, 23.31, 23.14, 19.75, 19.57; HRMS: calcd for $[C_{71}H_{107}N_{13}O_{11}+H]^{2+}$: 659.91793, found: 659.91815. RP-HPLC: 10–90%, R_t = 9.19 min.

Compound **1k**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (36 μmol) was coevaporated twice with dichloroethane and redissolved in MeOH (3 mL). First Et_3N (1 equiv, 5 μL) was added, then benzaldehyde (1.3 equiv, 5 μL). The reaction mixture was concentrated under reduced pressure for 2 h. The residue was taken up in MeOH (2 mL), put under argon and cooled to 0 °C. Sodium borohydride was added and stirring was continued for 3 h (LC/MS indicated completion). After evaporation of MeOH the residue was partitioned between EtOAc and aq $NaHCO_3$. The layers were separated and the organics were dried over $MgSO_4$, filtered and evaporated. Treatment with DCM/TFA (1:1 v/v, 1 mL, 1 h), concentration in vacuo and HPLC purification, amine **1k** was obtained in 15% yield (5 μmol, 8 mg).

^{13}C NMR (151 MHz, MeOH) δ 173.62, 172.85, 172.59, 162.64, 162.51, 137.01, 131.25, 130.51, 129.78, 129.60, 128.73, 128.57, 62.07, 62.00, 60.49, 56.07, 52.58, 51.58, 47.88, 42.12, 42.08, 40.68, 32.04, 30.87, 30.69, 27.81, 25.75, 23.31, 23.15, 19.74, 19.55; HRMS: calcd for $[C_{67}H_{99}N_{13}O_{11}+H]^{2+}$: 623.88917, found: 623.88892. RP-HPLC: 10–90%, R_t = 8.24 min.

Compound **1l**: compound **9** (50 mg, 36 μmol) was dissolved in EtOH (2 mL) and ammonium formate (23 mg, 360 μmol) was added. The solution was purged with argon and 10% Pd/C (20 mg) was added. The resulting suspension was stirred for 1 h and filtered through a double Whatman filter. The solution was concentrated and coevaporated with ethanol to give compound **10** that was used without further purification. Compound **10** (30 μmol) was dissolved in DMF (1 mL) and put under argon atmosphere. TBAI (5 equiv, 54 mg) and Na_2CO_3 (5 equiv, 16 mg) were added, followed by benzyl bromide (5 equiv, 18 μL). The mixture was stirred for 72 h, neutralised with 1 N HCl and concentrated under reduced pressure. The residue was taken up in DCM (1 mL) and TFA was added. After 1 h, the mixture was concentrated and subjected to HPLC purification to give **1l** (21%, 8 μmol, 9 mg).

^{13}C NMR (151 MHz, MeOH) δ 173.98, 173.62, 173.57, 173.47, 172.81, 172.52, 162.66, 162.44, 140.24, 137.00, 131.07, 131.07, 130.50, 130.50, 129.78, 129.78, 129.65, 129.65, 128.57, 128.00, 128.00, 127.78, 127.78, 124.24, 114.11, 62.07, 61.93, 60.48, 60.48, 56.22, 52.58, 51.57, 48.06, 47.83, 42.15, 42.07, 40.66, 32.07, 32.01, 30.89, 30.71, 25.76, 24.72, 24.66, 24.53, 23.28, 23.14, 19.74, 19.54; HRMS: calcd for $[C_{74}H_{105}N_{13}O_{10}+H]^{2+}$: 668.91264, found: 668.91260. RP-HPLC: 10–90%, R_t = 9.66 min.

Compound **1m**: Under an argon atmosphere, compound **10** (42 μmol) in DMF (2 mL) was cooled to 0℃. Subsequently Na_2CO_3 (5 equiv, 22 mg) and MeI (10 equiv, 26 μL) were added and stirring was continued for 72 h. The reaction mixture was quenched with 1 N HCl and concentrated under reduced pressure. The residue was taken up in DCM (1 mL) and TFA (1 mL) was added. After 1 h, the mixture was concentrated and subjected to HPLC purification to give **1m** (30%, 13 μmol, 15 mg).

^{13}C NMR (151 MHz, MeOH) δ 173.79, 173.64, 173.56, 173.53, 173.10, 172.87, 172.85, 172.60, 172.53, 147.79, 140.35, 136.98, 132.58, 130.50, 129.78, 128.58, 121.45, 62.23, 62.08, 60.52, 60.49, 57.75, 56.07, 55.41, 52.59, 51.58, 51.51, 48.29, 48.01, 42.10, 42.01, 40.69, 40.59, 37.39, 36.16, 32.11, 32.04, 30.97, 30.90, 30.87, 30.72, 25.76, 25.71, 24.73, 24.62, 24.54, 23.35, 23.29, 23.15, 23.09, 19.74, 19.56; HRMS: calcd for $[C_{63}H_{100}N_{13}O_{10}]^{+}$: 1198.77106, found: 1198.77192. RP-HPLC: 10–90%, R_t = 6.93 min.

4.4. Crystallisation

Compound **1c**: colourless prism-shaped crystals were obtained after slow evaporation of 2 μL droplets of 9.6 mg/mL peptide in 50% solution of MeOH in H_2O plus 2 μL of 0.2 M $Mg(OAc)_2$ in MeOH under paraffin oil in Terasaki plates.

Compound **1g**: colourless prism-shaped crystals were obtained after slow evaporation of 2 μL droplets of 10.7 mg/mL peptide in 80% solution of MeOH in H_2O plus 2 μL of 0.2 M $Mg(OAc)_2$ and 0.1 M Tris in MeOH under paraffin oil in Terasaki plates.

4.4.1. Crystal structure determination of 1c and 1g

A crystal was mounted in air and then rapidly transferred to liquid nitrogen. Synchrotron data were collected at beamline ID14-2 at the ESRF (Grenoble, France). Images were collected with DNA software,[15] processed with MOSFLM[16] and scaled with POINTLESS and SCALA.[17] Initial phases were obtained with ACORN,[18] using a mixed molecular replacement/ab initio procedure. As a starting fragment, the Leu-^{D}Phe-Pro-Val β-turn of native gramicidin S was used.[4f] ACORN produced suitable electronic density maps and by

Table 3
Selected crystallographic data for compounds **1c** and **1g**

	1c	1g
Formula	$5(C_{68}H_{101}N_{13}O_{11})\cdot O_{2.5}$	$5(C_{63}H_{99}N_{13}O_{11})\cdot O_{10}$
Formula weight	6423.10	6232.75
Wavelength (Å)	0.939	0.939
Crystal system	Monoclinic	Monoclinic
Space group	*I*2	*I*2
a (Å)	27.753(16)	27.853(4)
b (Å)	38.088(23)	38.274(3)
c (Å)	43.788(14)	42.610(3)
α (°)	90	90
β (°)	98.16(5)	96.765(2)
γ (°)	90	90
Cell volume (Å^3)	45,807(81)	45,108(8)
ρ_{calc} (g/cm^3)	0.931	0.918
No. form. units *Z*	4	4
μ (mm^{-1})	0.064	0.065
F(0 0 0)	13,840	13,440
Crystal size (mm^3)	0.15 × 0.10 × 0.10	0.24 × 0.18 × 0.10
T (K)	100(2)	100(2)
θ range (°)	2.71–25.27	2.70–25.26
Unique reflections	17,224	17,249
Measured reflections	40,732	47,126
Completeness (%)	94.2	95.5
Redundancy	2.4	2.7
$R_{(int)}$	0.074	0.047
Data in refinement	16,363	16,375
Data with $F_o > 4sig(F_o)$	11,065	15,335
Av. I/sig(I)	12.17	15.50
H, *K* and *L* min	−25, 0, 0	−25, 0, 0
H, *K* and *L* max	24, 34, 39	25, 34, 38
No. parameters	2494	2554
Extinction coef.	0.0230	0.0109
wR_2	0.8014	0.6112
R_1(obs data)	0.4076	0.2576
R_1 (all data)	0.4342	0.2620
Goof = S	4.1420	3.4210
Δ*F* peak/hole (e Å^{-3})	0.63/−0.47	0.62/−0.40
Mean residual	0.0000	0.0000
Rms deviation	0.1100	0.0900

means of COOT[19], five different gramicidin molecules could be traced in both asymmetric units. The structure of **1c** was refined by conjugate-gradient least-squares (CGLS) methods on F^2 with SHELXL[20] included in the WinGX[21] package, while the structure of **1g** was refined by full-matrix least-squares methods on F^2. All hydrogen positions were calculated and refined using a riding atom model. There are five crystallographically independent molecules per asymmetric unit in both crystal structures and there are several disordered parts in all molecules. This extensive degree of disorder in both structures impeded anisotropic refinement. Therefore, all atoms were refined using isotropic displacement parameters and with geometry restraints. Selected crystallographic data is reported in Table 3. Final figures were created using PyMOL (Delano Scientific, Palo Alto CA, USA); crystallographic data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif (accession numbers are CCDC-737680 for **1c** and CCDC-737679 for **1g**).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.07.042.

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