Total Synthesis of a Reversed-Bacicyclin Using a Combination of Solid- and Solution-Phase Methods

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Abstract: *Bacicyclin is a cyclic hexapeptide with antibacterial activity against* Enterococcus faecalis *and* Staphylococcus aureus *with minimum inhibition concentration (MIC) values of 8 and 12 μM, respectively. Studies on a reversed sequence of bacicyclin were conducted to investigate how the reversed peptide sequence affects its biological properties. A reversed-bacicylin, cyclo-(Gly-Leu-Val-Ile-Ala-Phe), was successfully synthesized by constructing the linear precursor on 2-chlorotrityl chloride resin using a Fmoc-based strategy. The HATU/HOAt reagent was applied in all peptidic bond formations, and the desired linear hexapeptide (82% yield) was cleaved off the resin using a mixture of trifluoroacetic acid:dichloromethane (2:8). The linear peptide was cyclized using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium-3-oxidehexa-fluorophosphate (HATU) as a coupling agent and diisopropylethylamine (DIPEA) as the base in a very dilute solution (0.001 M) in dichloromethane, then purified by octadecyl silica gel (ODS) column chromatography to obtain the reversed-bacicyclin (43.7% yield). The purity of the cyclic product was analyzed using analytical RP-HPLC (t_R = 20.01 min), and characterized by HR-TOF-MS, ^{1}H-NMR, and ^{13}C-NMR. The biological activity of the reversed-bacicyclin is much lower compared to bacicyclin, indicating that the amino acid sequence of the cyclopeptide dictates the antibacterial activity. This finding gives additional information on the relationship between peptide sequence and biological properties.*

Keywords: *bacicyclin; solid-phase peptide synthesis; cyclisation; cyclic hexapeptide; antibacterial peptide*

■ INTRODUCTION

Peptides, in particular cyclic peptides, have attracted much attention over the last few decades [1] because they exhibit unique advantages over linear peptides, such as their high affinity, fixed geometry and rigid structure, metabolic stability, and defined conformation. Cyclic peptides are a promising source of new drug candidates as they possess interesting biological activities, including antibiotic, antifungal [2], Cushing's disease [3], and anti-inflammatory [4]. Bacicyclin [cyclo-(D-Phe-D-Ala-Ile-Val-Ile-Gly)] (**1**) (Fig. 1) is a cyclic hexapeptide first isolated by Wiese et al. [5] from a marine *Bacillus* sp. strain (BC028). It shows antibacterial activity against the clinically relevant *Enterococcus faecalis* and *Staphylococcus aureus* with MIC values of 8 and 12 μM, respectively [5]. Structurally, its cyclic structure and the presence of D-configured residues make bacicyclin resistant to proteases, thus, it has potential as a drug

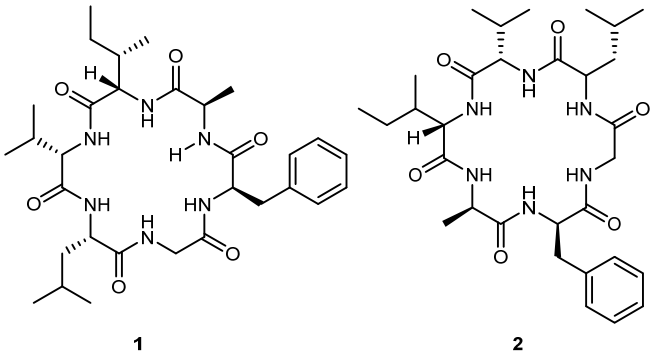


Fig 1. Structure of bacicyclin (**1**) and reversed-bacicylin (**2**)

candidate. Chen et al. [6] successfully synthesized bacicyclin and its analogs using a combination of solid- and solution-phase methods. However, synthetic bacicylin and analogs showed no significant antibacterial properties with an IC_{50} > 128 μM. The difference in antibacterial properties between the synthetic bacicylin and its natural product was caused by the different conformation and purities.

According to Damjanovic et al. [7], cyclic peptides with the same amino acid composition but different sequences exhibit different structural behavior in solution, which may change their activities. Rezai et al. [8] and Claro et al. [9] describe how cyclic peptides interact with the biological membrane and show that the interaction depends on the sequence, which dictates the conformation of the cyclic peptide. Therefore, it is interesting to compare the biological activities of peptides with the same amino acid composition but different sequences. Herein, we report the total synthesis of a bacicyclin isomer with a reversed sequence, cyclo-(Gly-Leu-Val-Ile-D-Ala-D-Phe) (**2**) (Fig. 1).

Most cyclic peptides are prepared via solid-phase peptide synthesis (SPPS) of the linear precursor, continued by macrocyclization on-resin or in solution [10]. The efficiency of the solution and on-resin cyclization were compared extensively by Sewald and colleagues [11] for the synthesis of cyclopeptides and cyclohexapeptides. The results showed that better method was achieved when cyclization was performed in solution-phase (9–36%) than on-resin (1–22%). A combination of solid- and solution-phase methods are commonly used for the synthesis of many cyclic peptides such as wollamide A, B, desotamide B [12], and their analogs [13-14]. This method was also used in the current synthesis, with SPPS and a Fmoc strategy performed to synthesize the linear precursor and cyclization conducted in solution-phase. The cyclization of a linear peptide with six residues is challenging [14], but the presence of two D amino acids facilitates the cyclization of the linear peptide, so D-Phe was located at the C-terminus and Gly at the N-terminus for cyclization [15]. The antibacterial properties of the synthesized reversed-bacicyclin against *S. aureus* and *E. faecalis* were then evaluated.

■ EXPERIMENTAL SECTION

Materials

The chemicals used were 2-chlorotrityl chloride resin, dimethylformamide (DMF), dichloromethane (DCM), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium-3-oxide hexafluorophosphate (HATU), *n*-hexane *N*,*N*-diisopropylethylamine (DIPEA), 1-hydroxy-7-azabenzotriazole (HOAt), piperidine, ethyl acetate, and trifluoroacetic acid. All amino acid residues, Fmoc-D-Phenylalanine, Fmoc-D-Alanine, Fmoc-L-Isoleucine, Fmoc-L-Valine, Fmoc-L-Leucine, Fmoc-L-Glycine, and 2-chlorotrityl chloride (0.972 mmol/g) were purchased from GL-biochem Ltd., Shanghai, China.

Instrumentation

Analysis of the linear and cyclic hexapeptides was performed on a Waters 2998 Photodiode Array Detector (PDA) with a wavelength of 210, 240, and 254 nm and LiChrospher 100 C-18 column (5 μm) for RP-HPLC. Acetonitrile (A) and deionized water (B) were used as the mobile phase with gradient elution and the addition of 0.1% trifluoroacetic acid (TFA) (vol/vol), with a flow rate of 1.0 mL/min and column temperature 25 °C for 30 min. The peptides were characterized by ^{1}H- and ^{13}C-NMR spectra using an Agilent ^{1}H-NMR 500 MHz and ^{13}C-NMR 125 MHz with deuterated solvent. The mass spectra were obtained from Waters HR-ToF-MS Lockspray, and the absorbance of loaded resin was measured on a UV-Vis Spectrophotometer (TECAN Infinite Pro 200).

Procedure

Synthesis of linear hexapeptides, a precursor of reversed-bacicyclin

The synthesis was performed on 2-chlorotrityl chloride resin (400 mg, 0.4 mmol), which was swollen in dichloromethane (10 mL) for 15 min at room temperature. Fmoc-D-Phe-OH (210 mg, 0.7 mmol) was loaded onto the resin in a mixture of dichloromethane (5 mL) and DIPEA (210 µL, 1.2 mmol). To measure the loading resin absorbance, 20% piperidine in DMF (3 mL) was added to 0.8 mg Fmoc-D-Phe-resin in an Eppendorf tube and left for 1 h, followed by sonification for 5 min before the absorbance was measured at 290 nm. Then, the resin was capped by adding 10 mL of MeOH:DCM:DIPEA (15:80:5) twice before the addition of 20% piperidine in DMF (5 mL) for 2 × 5 min to eliminate the Fmoc group, yielding a free amino group on the resin. Fmoc-D-Phe-NH_2 was coupled with the second residue, Fmoc-D-Ala-OH, using a combination of HATU (222.4 mg, 0.5 mmol) and HOAt (79.6 mg, 0.5 mmol) as a coupling agent and DIPEA (271.7 µL, 2.1 mmol) as a base in DMF (5 mL) for 4 h at room temperature. The Fmoc group was removed from Fmoc-D-Phe-D-Ala-Fmoc using 20% piperidine in DMF (5 mL) for 2 × 5 min to afford the resin-D-Phe-D-Ala-NH_2. This cycle of coupling and Fmoc deprotection was repeated with subsequent Fmoc-protected amino acids to obtain the resin-D-Phe-D-Ala-Ile-Val-Leu-Gly-NH_2. Finally, the peptide was cleaved from the resin using 20% TFA in dichloromethane (10 mL) for 2 × 20 min. After the collection of filtrate and subsequent TFA evaporation, the crude peptide was repeatedly washed with dichloromethane and dried under a vacuum. The linear peptide was injected into an analytical RP-HPLC (5–40% acetonitrile in water for 20 min, flow rate 1 mL/min, λ 240 nm) and characterized using HR-ToF-MS, ^{1}H-NMR (500 MHz, DMSO-d_6), and ^{13}C-NMR (125 MHz, DMSO-d_6).

Linear hexapeptide (precursor of reversed-bacicyclin): White solid; (91.3 mg, 82% yield); ^{1}H-NMR (500 MHz, DMSO-d_6, δ, ppm) 4.09 (m, CH-α, 2H), 4.38 (t, J = 8.4 Hz, CH- α, 1H), 4.12 (dd, J = 10.8;6.2 Hz, CH-α, 1H), 3.57 (d, J = 8.4 Hz, CH-α, 1H), 4.31 (m, J = 8.8 Hz, CH-α, 1H), 4.48 (q, J = 11.4; 4.2 Hz, CH-α, 1H), 8.48 (d, NH, 1H), 8.02 (d, NH, 1H), 7.94 (d, NH, 1H), 8.16 (d, NH, 1H), 7.83 (d, NH, 1H), 1.42 (s, NH_2, 2H), 1.57 (m, H-β/β' Leu, 2H), 1.64 (m, H-γ Leu, 1H), 0.81 (d, J = 7.8 Hz, H-δ Leu, 3H), 0.82 (d, J = 7.7 Hz, H-δ' Leu, 3H), 1.70 (m, H-β Val, 1H), 0.86 (d, J = 6.4 Hz, H-γ Val, 3H), 0.88 (d, J = 6.4 Hz, H-γ' Val, 3H), 1.97 (m, H-β Ile, 1H), 0.79 (d, H-γ Ile, 2H), 1.01 (d, H-γ' Ile, 3H), 0.76 (d, H-δ Ile, 3H), 1.13 (d, J = 8.4 Hz, H-β Ala, 3H), 3.06/2.93 (m, H-β/β' Phe, 2H), 7.26 (m, H-Bz-o, 2H), 7.22 (m, H-Bz-m, 2H), 7.16 (m, H-Bz-p Phe, 2H).^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm) 171.9 (C=O Gly), 172.7 (C=O Leu), 170.7 (C=O Val), 170.3 (C=O Ile), 171.5 (C=O Ala), 165.4 (C=O Phe), 41.4 (C-α Gly), 50.9 (C-α Leu), 58.0 (C-α Val), 57.0 (C-α Ile), 47.6 (C-α Ala), 53.5 (C-α Phe), 39.1 (C-β/β' Leu), 24.3 (C-γ Leu), 23.0 (C-δ Leu), 21.7 (C-δ' Leu), 30.7 (C-β Val), 18.5 (C-γ/γ' Val), 35.8 (C-β Ile), 15.2 (C-γ Ile), 29.9 (C- γ' Ile), 10.9 (C-δ), 18.5 (C-β Ala), 36.5 (C-β/β' Phe). HR-TOF-MS m/z 619.3892 $[M+H]^+$ (calcd. $C_{31}H_{51}N_6O_7$ 619.3894).

Synthesis of cyclic hexapeptides

The linear hexapeptide (30 mg, 0.05 mmol) was dissolved in DMSO (500 µL, 6.4 mmol), then dichloromethane (50 mL) before the addition of HATU (6 equiv. 110.7 mg, 0.3 mmol) and DIPEA (12 equiv. 75.3 µL, 0.6 mmol). The reaction mixture was stirred for 48 h at room temperature (monitored by TLC), then evaporated under vacuum to yield the crude cyclic product as a dark-yellow oil which was extracted between ethyl acetate (50 mL) and brine solution (3 × 30 mL). The organic fractions were combined and evaporated to give crude peptides as a bright-yellow solid, which were purified by reversed-phase ODS column chromatography (MeOH:H_2O = 5:5–6:4) to obtain the desired product (15.6 mg; yield 52%).

Reversed-Bacicyclin (**2**): White solid; 43.7% yield. ^{1}H-NMR (500 MHz, CD_3OD, δ, ppm) and ^{13}C-NMR (125 MHz, CD_3OD, δ, ppm) data can be seen in Table 1. HR-TOF-MS m/z $[M+H]^+$ 601.3713 (calcd. $C_{31}H_{49}N_6H_6$ 601.3714).

Antimicrobial assays

The antimicrobial activity of the test peptides was assessed by the disc method described by Mustafa et al.

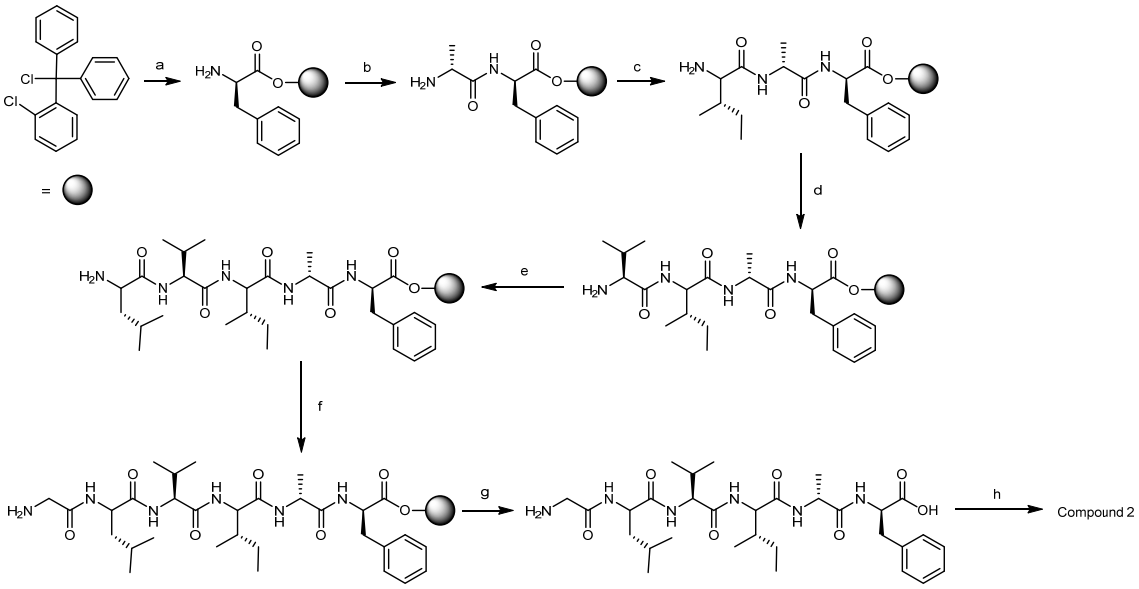
[16] with some modifications. Briefly, agar plates were inoculated with *Escherichia coli* (Gram-negative bacteria), *Enterococcus faecalis*, *Staphylococcus aureus* (Gram-positive bacteria) and *Candida albicans* (fungi), then sterile paper discs saturated with the test peptides were placed on top of the agar and incubated at 37 °C for 16–18 h before the resulting inhibition zones were measured.

The microdilution method described by Mustafa et al. [16] with some modifications was also performed to evaluate the antibacterial activity of the test peptides against *S. aureus* and *E. faecalis*. Briefly, the peptides were dissolved in 2% DMSO at a concentration of 1 µg/mL to prepare a series of serial dilutions (1000; 500; 250; 125; 62.5; 31.25; 15.62; 7.81; 3.90; 1.95; 0.97 and 0.48 ppm). The sample solutions, amoxicillin, and 2% DMSO were placed in a 96-well microplate and incubated at 37 °C for 18 h before the absorbance was measured at 600 nm to calculate the MIC values.

■ RESULTS AND DISCUSSION

The synthesis procedure of reversed-bacicyclin is shown in Scheme 1. The linear hexapeptide was prepared via an SPPS method using 2-chlorotrityl chloride (2-CTC) resin because the resin can suppress diketopiperazine formation due to the bulky size of the chlorotrityl group and provide a mild acidic cleavage condition. The synthesis was initiated by the loading of the first residue, Fmoc-D-Phe-OH, onto the resin to obtain 0.45 mmol/g, which was categorized as good (0.3–0.6 mmol/g) [17-18]. The unreacted sites were then capped using MeOH:DIPEA:DCM (15:5:85), and the Fmoc group was deprotected with 20% piperidine in DMF to obtain a free amino group. The product was then coupled with Fmoc-D-Ala-OH using HATU/HOAt as the coupling reagent, and DIPEA as the base before the peptide was elongated by the attachment of subsequent L-isoleucine, L-valine, L-leucine, and glycine residues and final Fmoc deprotection to give the linear hexapeptidyl resin-D-Phe-D-Ala-Ile-Val-Leu-Gly-NH_2 (**2**).

The linear hexapeptide was cleaved from the resin using 20% TFA in DCM to yield a high purity linear product for macrocyclization. The HPLC chromatogram showed a single peak with a retention time of 7.140 min (Fig. 2), and the molecular ion peaks at *m/z* 619.3892 $[M+H]^+$ and $[2M+H]^+$ 1237.8109 in the HR-TOF-MS spectra confirmed the successful synthesis of the desired hexapeptide (Fig. 3). The ^{1}H- and ^{13}C-NMR of the linear precursor revealed five amide NH at



(a) (1) Fmoc-D-Phe-OH, DIPEA, in dichloromethane, 8 h, rt, 20% piperidine in DMF, (2) MeOH:DIPEA:DCM (15:5:80), 2 × 20 min, rt, (b) (1) Fmoc-D-Ala-OH, HATU/HOAT, in DMF, 4 h, rt, (2) 20% piperidine in DMF, (c) (1) Fmoc-L-Ile-OH, HATU/HOAT, in DMF, 4 h, rt, (2) 20% piperidine in DMF, (d) (1) Fmoc-L-Val-OH, HATU/HOAT, in DMF, 4 h, rt, (2) 20% piperidine in DMF, (e) (1) Fmoc-L-Leu-OH, HATU/HOAT, in DMF, 4h, rt, (2) 20% piperidine in DMF, (f) (1) Fmoc-L-Gly-OH, HATU/HOAT, in DMF, 4 h, rt, (2) 20% piperidine in DMF, (g) 20% TFA in DCM, 2 × 20 min, rt, (h) HATU, DIPEA, in DCM, 10^{-3} M, 48 h, rt.

Scheme 1. Fmoc-based SPPS and solution-phase macrocyclization of reversed bacicyclin

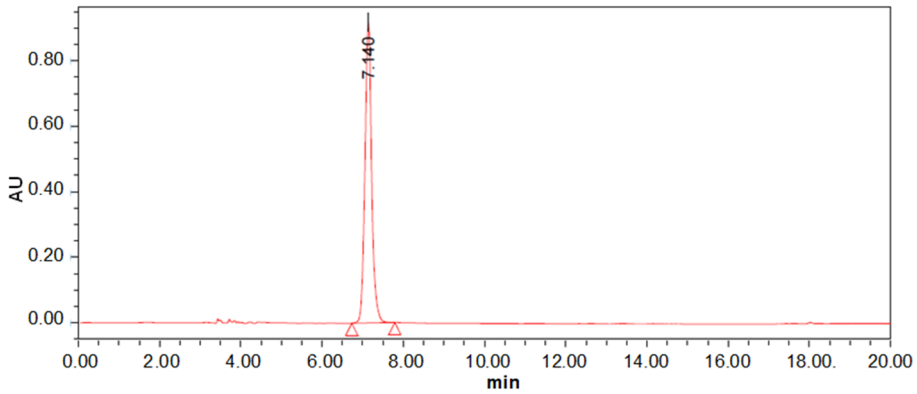


Fig 2. HPLC spectra of linear hexapeptide (precursor of bacicyclin) in acetonitrile:H_2O (5–40% linear gradient) at a flow rate of 1 mL/min, and λ 240 nm

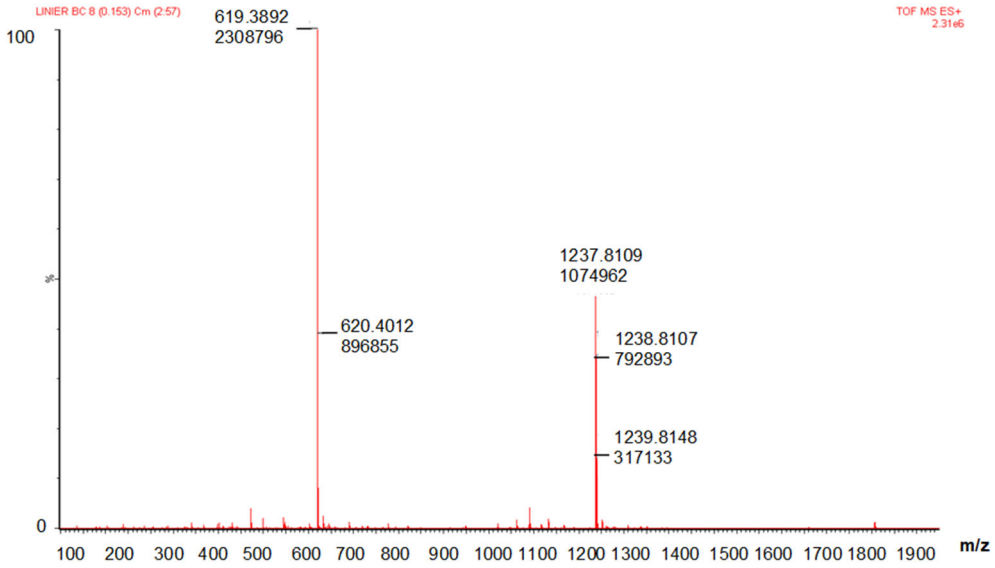


Fig 3. MS spectra of the linear hexapeptide

8.48 (d, 1H), 8.02 (d, 1H), 7.94 (d, 1H), 8.16 (d, 1H), and 7.83 (d, 1H), six α-protons at the chemical shifts of 4.09 (1H), 4.38 (1H), 4.12 (1H), 3.57 (1H), 4.31 (1H), and 4.48 (1H) ppm, one NH_2 proton at the chemical shift of 1.42 (s, 2H) ppm, six carbonyls at the chemical shifts of 165.4 (Gly), 172.7 (Leu), 170.7 (Val), 170.3 (Ile), 171.5 (Ala), and 171.9 (Phe) ppm and six α-carbons at the chemical shifts of 41.4, 50.9, 58.0, 57.0 47.6, and 53.5 ppm. Taken together, these data confirm the peptidic structure of the linear hexapeptide and 82% yield without any further purification required.

The linear hexapeptide was less soluble in DCM, DMF, acetonitrile, and other organic solvents but dissolved well in DMSO. Therefore, to avoid difficulties with DMSO removal, a minimal amount of DMSO was used to dissolve the peptide. Macrocyclization was firstly trialed in a dilute solution of 0.001 M in dichloromethane following the protocol of Ma and colleagues [19], but the linear peptide was still detected in the MS spectra. Therefore, the amount of HATU (6 equiv.) was increased, and the addition of DIPEA was stopped until it reached 12 equivalent. The reaction was completed in 48 h, yielding approximately 53% of the desired compound. The reaction was monitored by TLC (*n*-hexane:isopropanol (7:3) and peptide dimerization was avoided by conducting the macrocyclization reaction at a very dilute peptide concentration (less than 1 mM).

The success of the cyclization relies on the ring size

and the residues present in the peptide sequence, with side reactions, such as racemization, occurring if the linear peptide consists of less than seven amino acid residues [20]. However, the presence of amino acids such as proline, D-configured amino acids, the thiazole or oxazole ring, and an achiral amino acid such as glycine can increase cyclization success [21]. To minimize racemization, thus diastereomer formation during cyclization, the cyclic target was disconnected at the site between D-phenylalanine at the C-terminus and glycine at the N-terminus, which can also reduce steric resistance during the macrocyclic process.

HPLC analysis of the crude product showed that the cyclic peptide was the major product without any remaining linear starting material in the reaction mixture. The mixture was then concentrated and extracted with sodium chloride solution and evaporated using a rotary evaporator. Crude bacicyclin was purified by a flash column chromatography using MeOH:H_2O (gradient: 6:4-5:5) as the eluent, yielding 15.6 mg of compound **2** as white solid (52%). The purity of peptide **2** was checked by analytical RP-HPLC (Fig. 4; retention time = 20.01 min), and it was characterized by HR-TOF-MS, ^{1}H-NMR, ^{13}C-NMR, and IR. The mass spectra of HR-TOF-MS showed the correct molecular ion peak of the desired cyclic peptide with *m/z* 601.3713 $[M+H]^+$ (calcd. *m/z* 601.3714) and *m/z* 623.3525 $[M+Na]^+$ (calcd. *m/z* 623.3526) (Fig. 5).

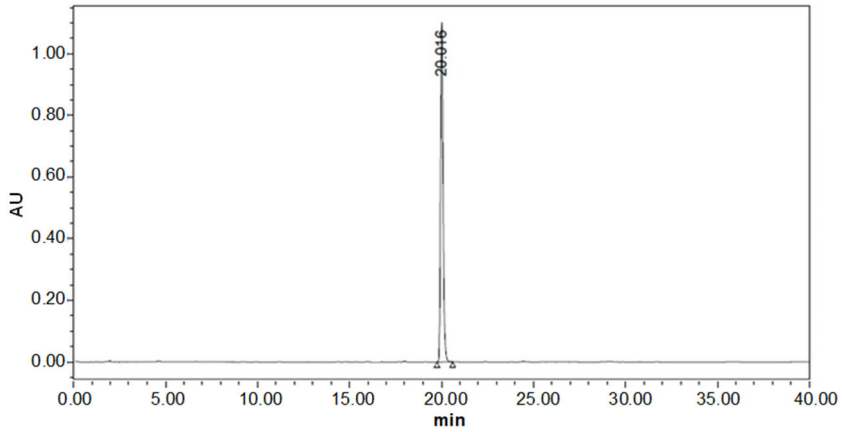


Fig 4. Analytical RP-HPLC chromatogram of reversed-bacicyclin 2 in acetonitrile:water (5–40% linear gradient) at a flow rate of 1 mL/min and λ 240 nm

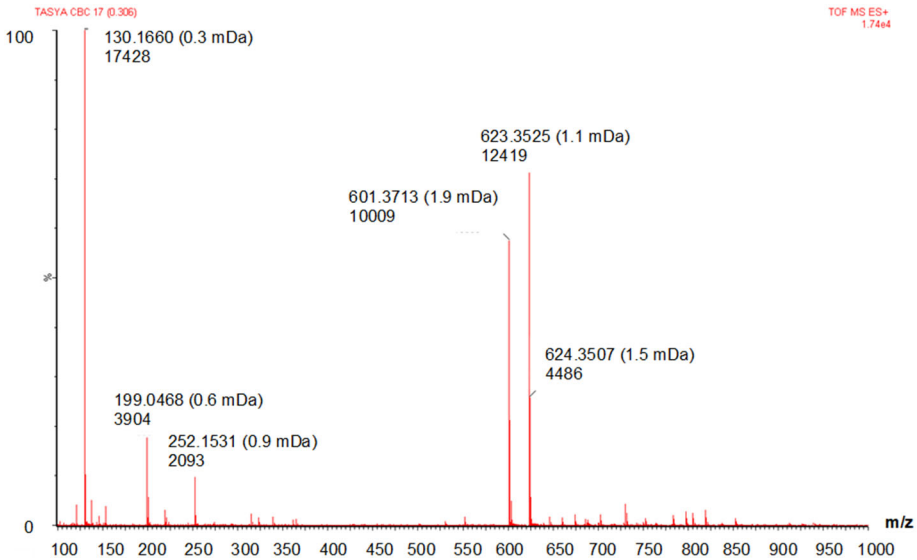


Fig 5. MS spectra of reversed-bacicyclin 2

Table 1. ^{1}H-NMR and ^{13}C-NMR spectra data of reversed-bacicyclin and comparison with bacicyclin spectral data by Wiese and colleagues

Amino acid residues	δ_C **1** [5] (CD_3OD, 150 MHz)	δ_C **2** (CD_3OD, 125 MHz)	δ_H **1** [5] (CD_3OD, 600 MHz)	δ_H **2** (synthesized) (CD_3OD, 500 MHz)
Gly				
CO	179.1	175.3		
α	41.4	41.2	3.36, t (*J* = 8.4 Hz)	3.38, t (*J* = 8.5 Hz)
Leu				
CO	171.5	172.0		
α	52.7	53.2	3.8, t (*J* = 8.4 Hz)	3.88, t (*J* = 8.5 Hz)
β/β’	41.8	41.9	1.58, m	1.60, m
γ	26.2	25.9	1.62, m	1.67, m
δ	22.5	23.2	0.91, d (*J* = 7.8 Hz)	0.99, d (*J* = 7.8 Hz)
δ'	21.6	21.7	0.96, d (*J* = 7.7 Hz)	1.08, d (*J* = 7.7 Hz)
Val				
CO	175.5	175.2		
α	60.6	62.6	4.21, dd (*J* = 10.8; 6.2 Hz)	4.22, dd (*J* = 10.5; 6.3 Hz)
β	31.8	32.0	1.98, m	1.86, m
γ	19.5	19.5	0.89, d (*J* = 6.4 Hz)	0.96, d (*J* = 6.4 Hz)
γ'	18.9	17.6	0.88, d (*J* = 6.6 Hz)	0.92, d (*J* = 6.8 Hz)
Ile				
CO	173.2	173.2		
A	58.9	58.7	4.19, d (*J* = 8.5 Hz)	4.19, d (*J* = 8.5 Hz)
B	36.1	37.3	2.03, m	2.13, m
Γ	12.1	12.1	0.84	0.85
γ'	27.5	26.1	1.28	1.28
Δ	14.6	16.1	0.86	0.86
Ala				
CO	173.4	174.1		
A	49.9	50.1	4.30, q (*J* = 9.0 Hz)	4.3, q (*J* = 8.8 Hz)
B	18.1	18.1	1.17, d (*J* = 8.4 Hz)	1.15, d (*J* = 8.5 Hz)
Phe				
CO	173.9	174.7		
A	56.8	55.8	4.3, q (*J* = 11.4; 4.2 Hz)	4.3, q (*J* = 11.0; 4.2 Hz)
β/ β’	29.1	28.1	3.31/3.05, m	3.20/3.16, m
Bz-I	128.9	139.0		
Bz-o	112.3	122.5	7.22, m	7.29, m
Bz-m	119.4	127.6	6.98, m	7.18, m
Bz-p	124.6	129.4	7.18, m	7.27, m

The ^{1}H-NMR and ^{13}C-NMR spectra of **2** and the linear peptide were different, with the absence of the chemical shift at 1.42 ppm of the free amine ($-NH_2$) proton of glycine in the ^{1}H-NMR spectra confirming cyclization (Table 1). The absence of NH_2 proton and the presence of NH amide signals in ^{1}H-NMR are commonly observed when the linear peptide is completely converted into a cyclic peptide [22-23]. Moreover, the successful formation of an amide bond between the amino group of glycine and carboxyl group

of D-phenylalanine was proven by the presence of the deshielding carbonyl signal of Phe at 175.3 ppm. Overall, the ^{13}C-NMR spectra displayed six amide-type carbonyls (δ 175.3, 172.0, 175.2, 173.1, 174.1, 174.7 ppm), five α-methine carbons (δ 53.2, 62.6, 58.7, 50.1, 55.8 ppm), α-methylene carbon (41.2), seven methyl groups (23.2, 21.7, 19.5, 17.6, 12.1, 16.1, 18.1 ppm), and three methylene groups (41.9, 37.3, 28.1 ppm). The NMR spectral data of **2** was similar to the NMR data obtained by Wiese et al. [5] for bacicyclin (Table 1).

The antimicrobial activities of the linear and cyclic peptides of **2** were assessed, showing that the linear and cyclic peptides of the reversed-bacicyclin **2** inhibited the growth of gram-negative *E. coli* with weak antibacterial activity at a concentration of 2000 ppm. The peptides also exhibited moderate antibacterial activity against *S. aureus* and *E. faecalis* but were not as effective as the natural product [5] or synthetic bacicylin [6], indicating that reversing the peptide structure affected the biological properties of the peptide.

■ CONCLUSION

Reversed-bacicyclin (cyclo-Gly-Leu-Val-Ile-D-Ala-D-Phe) has been successfully synthesized in a two-step process, achieving an overall yield of 43.7%. However, the biological properties of the reversed peptide were different to the natural product. This finding gives additional information on the relationship between peptide sequence and biological properties.

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■ AUTHOR CONTRIBUTIONS

RM, AF, and ATH designed the research. AF and KF conducted the research. TM, DH, N, and US helped in the purification step, RM and SI wrote the manuscript. All authors agreed to the final version of this manuscript.

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