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Synthesis of Nodupetide Analogue: Substitution of β- to α-hydroxy Acid

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Abstract

Nodupetide, a cyclodepsipeptide with the potential as an antimicrobial agent, was isolated from the fermentation of the fungi *Nodulisporium* sp strain IFB-A163. It possessed antibacterial activity against *Pseudomonas aeruginosa* and revealed an insecticidal activity. In our effort to synthesized nodupeptide, the preparation of the (3*S*,4*S*)-3-hydroxy-4-methylhexanoic acid (HMHA) as one of the nodupetide residue was found to be challenging, where it is not commercially available and the synthetic trial of the HMHA was not successful. This led us to synthesize nodupetide derivative by replacing HMHA with its α-hydroxy acid to give the analogue [(2*S*,3*S*)-Hmp]^2-nodupetide. A combination of solid- and solution-phase peptide synthesis was applied in the synthesis nodupetide analogue. Esterification on the resin for synthesis [(2*S*,3*S*)-Hmp]^2-nodupetide was achieved by placing the reaction at the last stage of the linear peptide synthesis and obtained in overall yield of 4.3 %. We also conducted antimicrobial activity screening for [(2*S*,3*S*)-Hmp]^2-nodupetide and its linear precursor, showing inactive activity. The alter of hydrophobicity on the nodupetide analogue has very important role in the difference of bioactivity compared to their parent compound.

Keywords: Nodupetide, [(2*S*,3*S*)-Hmp]^2-nodupetide, SPPS, Cyclopeptide, Antimicrobial

Introduction

Cyclic peptide is a promising antimicrobial agent that has tremendous potential to be developed. Cyclic peptides that have unnatural amino acid residues are known to enhance helicity (peptides with α-helical conformation), which leads to a more rigid conformation. This can contribute to increased cell permeability as structural stability of membrane interactions is increased.[1] In addition, the anchoring of unnatural amino acid residue is more resistant to proteolytic enzymes, thus in terms of pharmacokinetics is more favorable because it has a longer half-life in plasma [2].

Nodupetide (1) (**Figure 1**), a cyclodepsipeptide, was isolated from the scaled-up fermentation of the fungi *Nodulisporium sp* strain IFB-A163, which was originally isolated from the gut of the insect *Riptortus pedestris*. The distinctive structure of nodupetide is the existence of 1 unnatural amino acid residue, which is (3*S*,4*S*)-3-hydroxy-4-methylhexanoic acid (HMHA) that forms an ester bond (depside) with valine. Nodupetide has inhibitory activity against *Pseudomonas aeruginosa* and also insecticidal activity against *Nilaparvata lugens*. The structure of nodupetide consists of 4 proteogenic amino acids, which are [gly]^2, [val]^{3,6}, [ala]^5, and 2 non-proteogenic amino acids [HMHA]^1, [D-leu]^4 [3]. The uniqueness of the structure and potential biological activity possessed by nodupetide compounds provides an attraction for the exploration or development of synthetic nodupetide compounds and its analogues. Until now, any synthetic nodupetide and its analogues have never been reported.

The synthesis of cyclodepsipeptides in solid phase is known to possess many difficulties especially in the formation of ester bonds rather than amide bonds. The presence of depside bonds in the backbone is very susceptible to the formation of diketopiperazine (DKP) [4]. The combination of solid- and solution-phase peptide synthesis is an effective combination in the synthesis of cyclodepsipeptide and cyclopeptide. Linear peptides are synthesized in the solid phase, and cyclization is carried out in the solution phase [5]. Rahmadani and team applied a strategy to form a depside bond at the last step of the linear peptide synthesis stage with the aim to minimize the formation of DKP [4].

In this study, we developed a synthetic strategy for nodupetide analogue. The unavailability of HMHA commercially and the difficulties in developing HMHA synthesis strategies were led us to substitute HMHA into (2*S*,3*S*)-2-hydroxy-3-methylpentanoic acid ((2*S*,3*S*)-Hmp), which is an α-hydroxy carboxylic acid, to give [(2*S*,3*S*)-Hmp]^2-nodupetide. As a consequence, all starting materials are available and ready to be used at low cost to develop methodologies for the synthesis of nodupetide analogue. Thus, in this study we explained the combination of solid- and solution-phase peptide synthesis methodology for [(2*S*,3*S*)-Hmp]^2-nodupetide (2).

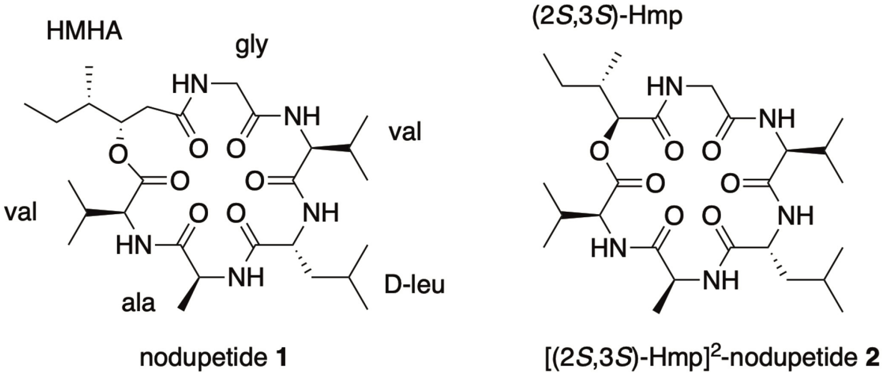

Figure 1 Structure of nodupetide (1) and its analogues [(2*S*,3*S*)-Hmp]^2-nodupetide (2).

Materials and methods

Materials

The peptides were characterized using ^{1}H-NMR and ^{13}C-NMR on Agilent NMR 500 MHz (^{1}H) and 125 MHz (^{13}C) using $CDCl_3$ solvent. Mass spectroscopy spectra were measured using Waters HR-ToF-MS Lockspray. The loading resin absorbance was measured on the TECAN Infinite pro 200 UV-Vis spectrophotometer. Analysis of linear and cyclic peptides was performed by Waters 2998, Photodiode Array Detector (PDA), LiChrospher 100, with C-18 column (5 µM, 4.6 mm x 250 mm, COSMOSIL) at the wavelength of 210, 240, and 254 nm. The mobile phase used a gradient eluent consisting of deionized water (H_2O) (A) with 0.1 % TFA and acetonitrile B with 0.1 % TFA. All amino acids, coupling reagents, and resin were purchased from GL-Biochem, Shanghai, China. They included 2-chlorotrityl chloride resin, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), N-[(dimethylamino)-1*H*-1,2,3-triazolo-4,5-[*b*]pyridine-1-yl-methelene]-*N*-methylmethanaaminium hexafluorophosphate *N*-oxide (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), Fmoc-L-ala-OH, Fmoc-gly-OH, Fmoc-D-leu-OH, Fmoc-L-val-OH, L-isoleucine. The other reagents such as dichloromethane (DCM), *N*,*N*′-dimethylformamide (DMF), *N*,*N*′-diisopropylethylamine (DIPEA), *N*,*N*′-

diisopropylcarbodiimide (DIC), 4-dimethylaminopyridine (DMAP), sodium nitrite, sodium sulphate, piperidine, methanol, *n*-hexane, ethyl acetate, acetic acid, sulphuric acid, and trifluoroacetic acid (TFA) were pro-analysis grade.

Synthesis of [(2S,3S)-Hmp]^2-nodupetide 2

Preparation of (2S,3S)-Hmp

Amino acid L-isoleucine (2 g, 15.25 mmol) was dissolved in 1 M sulfuric acid (40 mL), then sodium nitrite solution (6.31 g, 91.50 mmol) was added into the solution slowly at 0 °C for 2 h. The reaction mixture was stirred for 17 h at room temperature. The reaction was monitored using thin layer chromatography (propanol: Methanol: Acetic acid (7:2:1)). The reaction mixture was then added with sodium bicarbonate until the solution reached pH 2. Furthermore, the reaction mixture was saturated with sodium chloride and extracted using ethyl acetate (3×50 mL). The organic fraction was dried with anhydrous sodium sulphate, and filtered. The filtrate was concentrated to give a colorless oil of (2*S*,3*S*)-Hmp (1,022.6 mg; 51.1 %). HR-ToF-MS *m/z* [M-H]$^-$ 131.0709 (calcd. *m/z* 131.0708 for $C_6H_{11}O_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, δ_H ppm) δ 4.16 (d, 1H, *J* = 3.8 Hz, CHC*H*(OH)COOH), 1,91 (bs, 1H, CH_3C*H*CH_2),1.46-1.38 (m, 1H, CHC*H*HCH_3), 1.32-1.24 (m, 1H, CHCH*H*CH_3), 1.00 (d, 3H, *J* = 6.8 Hz, C*H*$_3$CH), 0.91 (t, 3H, *J* = 7.4 Hz, CH_3CH_2); ^{13}C-NMR (125 MHz, $CDCl_3$) δc 179.0, 74.8, 38.9, 23.8, 15.4, 11.8 (^{1}H-NMR and ^{13}C-NMR data were agreement with the previously describe) [6].

Solid phase peptide synthesis for linear [(2S,3S)-Hmp]^2-nodupetide

The 2-chlorotrityl chloride resin (0.99 mmol/g capacity) (400 mg, 0.6 mmol) was swollen in DCM (10 mL) for 15 min in room temperature. The first amino acid residue (Fmoc-L-ala-OH) (0.6 mmol) was attached onto the resin in dichloromethane (4 mL) and basic DIPEA (1.2 mmol) for 4 h. Absorbance measurement of loading resin was performed by incubating 0.5 mg of Fmoc-L-AA1-OH-resin in 20 % piperidine/DMF (3 mL) for 30 min and followed by sonification for 5 min. The loading resin was measured by UV-Vis spectrophotometry at 290 nm and resulting the loading resin value of 0.43 mmol/g. Furthermore, the resin was capped by adding (MeOH:DCM:DIPEA) (15:80:5) (10 mL) for 2×15 min. The Fmoc-peptidyl resin was deprotected using 20 % piperidine in DMF (5 mL) for 2×4 mL and a chloranil test was performed to determine the presence of free amine groups. Peptidyl-NH_2 resin was developed by adding Fmoc-amino acids (3 eq), HBTU (3 eq), HOBt (3 eq), and DIPEA (6 eq) in DMF (4 mL). The mixture was stirred for 4 h at room temperature and followed with Fmoc deprotection using 20 % piperidine in DMF. The cycle of coupling and deprotection was repeated to afford tetrapeptidyl resin. Furthermore, the fifth residue (2*S*,3*S*)-Hmp was added to tetrapeptidyl resin using HATU (3 eq), HOAt (3 eq), and DIPEA (6 eq) in DMF (4 mL) and stirred for 2×4 h. The next step, esterification was carried out by adding the sixth residue Fmoc-L-val-OH (7 eq) in DCM (4 mL) into the pentapeptidyl-OH resin and the reaction was moved into reaction tube. Then, a mixture of DIC (10 eq), DMAP (0.7 eq), and DIPEA (1.4 eq) was added into the reaction tube. The mixture was stirred for 3×5 h at 45 °C. After the reaction was completed, the resin was filtered and washed with DMF and DCM. Next, Fmoc deprotection was conducted by 20 % piperidine in DMF, and followed by chloranil test to confirm the presence of free amine group. The final step, the resin was cleaved using 20 % TFA in DCM (5 mL) and shaken for 2×10 min. The filtrate was collected and evaporated to obtain crude linear hexadepsipeptide, as a pale-yellow oil. The linear peptide was analyzed by analytical RP-HPLC (10 - 90 % acetonitrile in water with TFA 0.1 % for 45 min, flow rate 1 mL/min, λ 240 nm). The crude was purified by silica ODS column chromatography (MeOH:H_2O 6:4) to obtain linear peptide (white powder, 3.7 mg, yield 2.7 %). The purity of linear peptide was analyzed by analytical RP-

HPLC (10 - 90 % acetonitrile in water with TFA 0.1 % for 45 min, flow rate 1 mL/min, λ 240 nm) and characterized by HR-ToF-MS *m/z* 572.3660 $[M+H]^+$ (calcd. *m/z* 572.3659 for $C_{27}H_{50}N_5O_8$).

Cyclisation in solution phase

The crude linear hexadepsipeptide (25 mg, 0.04 mmol) was dissolved in DCM (50 mL) before the addition of HATU (3 eq, 49.84 mg, 0.13 mmol) and DIPEA (6 eq, 89.01 µL). The reaction mixture was stirred for 48 h at room temperature (monitored by TLC). The reaction mixture was concentrated to give crude cyclic product as a dark yellow oil, and then purified by column chromatography (*n*-hexane: Ethyl acetate 1:1) to afford cyclic peptide (2) (white powder, 2.2 mg, yield 4.3 %). The cyclic hexadepsipeptide was analyzed by analytical RP-HPLC (20 - 80 % acetonitrile in water for 40 min, flow rate 1 mL/min, λ 240 nm) and characterized by HR-ToF-MS *m/z* 554.3610 $[M+H]^+$ (calcd *m/z* 554.3609 for $C_{27}H_{48}N_5O_7$), ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$).

Biological assay

Microdilution method

The [(2*S*,3*S*)-Hmp]^2-nodupetide 2 and its linear were tested for their antimicrobial activity following microdilution method in a 96-well microplate with a Mueller-Hinton culture against 4 pathogen microbes (*C. albican*, *P. aeruginosa*, *S. aureus*, *E. coli*). The peptide was dissolved in 2 % DMSO to a concentration of 2,000 µg/mL and then dissolved gradually: 2,000; 1,500; 1,000; 500; 250; 125; 6.25; 31.25; 15.62; 7.81; 3.90; 1.95; 0.97, and 0.48 µg/mL. The peptides solution, ciprofloxacin, and 2 % DMSO were incubated in a 96-well microplate at 37 °C for 18 h. Furthermore, the plate was read in spectrophotometer at 600 nm, and the MIC was calculated as percentage of microbial growth inhibition.

Results and discussion

In solid phase peptide synthesis of the linear precursor [(2*S*,3*S*)-Hmp]^2-nodupetide, acid-labile 2-chlorotrityl chloride (2-CTC) resin was used as the solid support (**Figure 2**). The cyclization site was chosen between alanine and valine to place the esterification step at the last stage of the linear depsipeptide chain elongation, aiming to suppress DKP formation and hydrolysis during Fmoc deprotection and resin cleaved [4,7]. In addition, alanine as C-terminal provides an advantage in facilitating cyclization reaction [8,9].

Synthesis of the linear depsipeptide [(2*S*,3*S*)-Hmp]^2-nodupetide was initiated by anchoring the first amino acid Fmoc-L-alanine onto the resin. This step was conducted by using DIPEA as a base in dichloromethane. Next, capping of the resin is undertaken to mask the remaining chlorine groups on the resin to prevent early rupture of the peptide chain. All steps of the linear depsipeptide coupling reaction and Fmoc group deprotection were monitored using the chloranil test. The removal of Fmoc groups on the peptide resin was carried out using 20 % piperidine in DMF. The solid-phase peptide synthesis of tetrapeptidyl resin was carried out using combination of HBTU/HOBt coupling reagents. Subsequently, the coupling of the fifth amino acid residue (2*S*,3*S*)-Hmp onto the tetrapeptidyl resin, the coupling reaction was carried out using a combination of HATU/HOAt and performed as double coupling protocol to complete the amide bond formation. (2*S*,3*S*)-Hmp was already prepared from L-isoleucine by diazotation reaction with $NaNO_2$ under acidic conditions following the protocol of Rahmadani and co-workers [4]. Incorporating (2*S*,3*S*)-Hmp was generated free hydroxy group at the terminus of pentapeptidyl resin. Furthermore, the coupling of the sixth residue of Fmoc-L-val-OH onto the pentapeptidyl resin followed Rahmdani protocol [4]. The depside bond formation was carried out by Steglich esterification using DIC/DMAP with the addition of DIPEA for 3×5 h at 45 °C and finally afforded Fmoc-hexadepsipeptide resin. The Fmoc group was removed to give the hexadepsipeptide resin, which was ready to be cleaved from the resin by 20 % TFA in DCM and afforded crude linear hexadepsipeptide. Purification of the crude depsipeptide using silica ODS column chromatography was gave the desired linear depsipeptide, unfortunately in a low yield (2.7 %). The HR-ToF-MS spectra showed the correct molecular ion peak of linear [(2*S*,3*S*)-Hmp]^2-nodupetide *m/z* 572.3660 $[M+H]^+$ (calcd. *m/z* 572.3659 for $C_{27}H_{50}N_5O_8$).

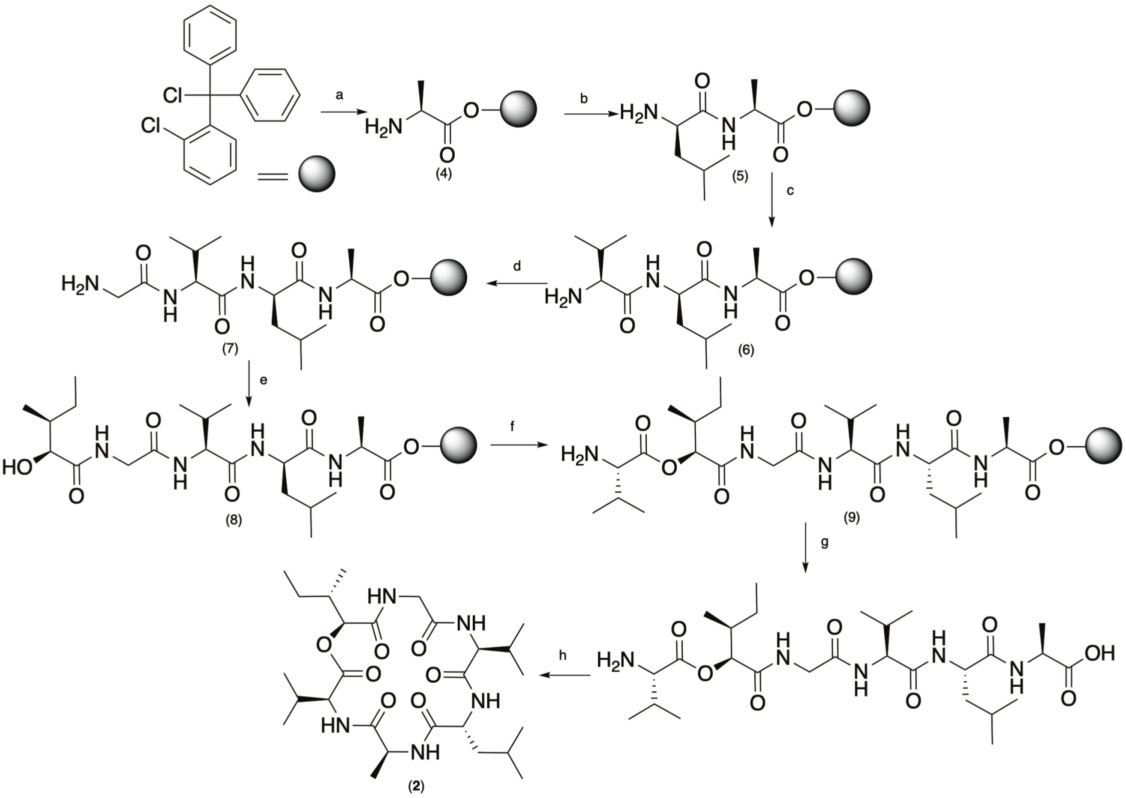

Figure 2 General procedure Fmoc-strategy SPPS and solution phase macrocyclization of 2, (a) (1) Fmoc-L-ala-OH, DIPEA, DCM, 4 h, rt; (2) MeOH/DCM/DIPEA (15:80:5); (3) 20 % piperidine in DMF, (b) (1) Fmoc-D-leu-OH, HBTU, HOBt, DIPEA, DMF, 4 h, rt; (2) 20 % piperidine in DMF, (c) (1) Fmoc-L-val-OH, HBTU, HOBt, DIPEA, DMF, 4 h, rt; (2) 20 % piperidine in DMF, (d) (1) Fmoc-gly-OH, HBTU, HOBt, DIPEA, DMF, 4 h, rt; (2) 20 % piperidine in DMF, (e) (1) (2*S*,3*S*)-Hmp, HATU, HOAt, DIPEA, DMF, 2×4 h, rt; (f) (1) Fmoc-L-val-OH, DIC, DMAP, DIPEA, DCM:DMF, 3×5 h, 45 °C, (2) 20 % piperidine in DMF, (g) 20 % TFA in DCM, 2×10 min, rt, (h) HATU, DIPEA, DCM, 48 h, rt.

Since the yield of the purified linear [(2*S*,3*S*)-Hmp]^2-nodupetide was very insufficient, cyclization of linear [(2*S*,3*S*)-Hmp]^2-nodupetide was performed using the crude linear. Cyclization of linear [(2*S*,3*S*)-Hmp]^2-nodupetide was carried out under highly dilute conditions (10^{-3} M) using HATU, to avoid the formation of side reaction. Purification of crude cyclic was carried out using silica column chromatography with eluent *n*-hexane:ethyl acetate and obtained white powder with overall yield of 4.3 % . [(2*S*,3*S*)-Hmp]^2-nodupetide was analyzed by analytical RP-HPLC and characterized by HR-ToF-MS, ^{1}H-NMR and ^{13}C-NMR. HR-ToF-MS showed correct molecular ion peak for the desired cyclodepsipeptide with *m/z* 554.3610 $[M+H]^+$ (calcd. *m/z* 554.3609 for $C_{27}H_{48}N_5O_7$) (**Figure 3**). The analytical RP-HPLC chromatogram was showed a single peak at retention time 28.65 min (**Figure 4**). A structural assignment of [(2*S*,3*S*)-Hmp]^2-nodupetide was achieved by ^{1}H-NMR and ^{13}C-NMR. The complete ^{1}H-NMR and ^{13}C-

NMR resonance assignment of [(2*S*,3*S*)-Hmp]^2-nodupetide is presented in (**Table 1**). The spectra of ^{1}H-NMR showed the existence of alpha protons at 4.71, 4.48, 4.13, 4.04, 3.69 ppm with total integration of 6 alpha protons. ^{13}C-NMR spectra showed the presence of 5 carbonyl signals of amides at 172.5, 171.2, 171.9, 170.5, 169.5 and 1 carbonyl signal of ester at 167.8 ppm. The presence of depside bond in the structure was confirmed by the presence of C-O *sp* chemical shift at 74.5 ppm [3]. The ^{1}H-NMR and ^{13}C-NMR data showed the number of protons and carbons that corresponded to the structure of [(2*S*,3*S*)-Hmp]^2-nodupetide.

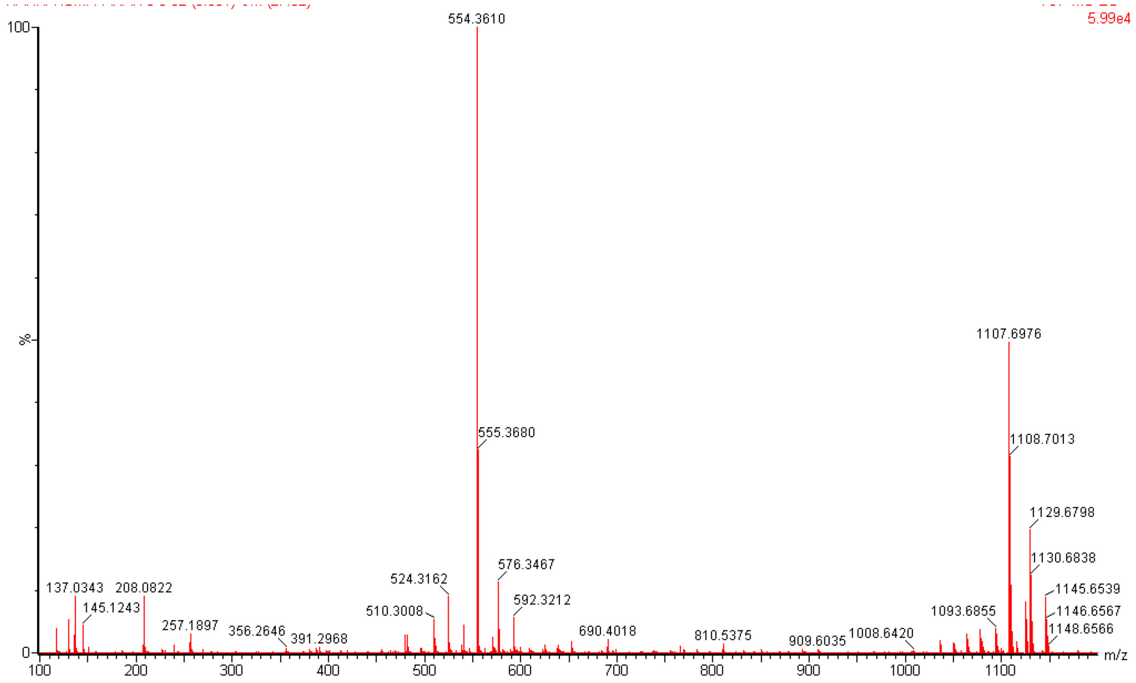

Figure 3 Mass spectrum of [(2S,3S)-Hmp]^2-nodupetide 2.

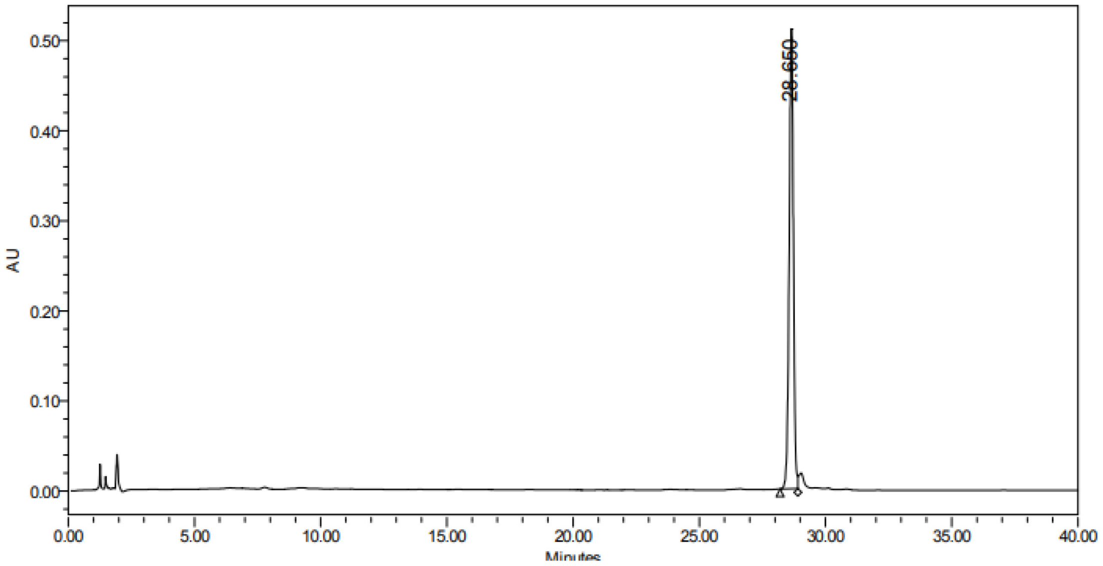

Figure 4 Analytical RP-HPLC chromatogram of [(2*S*,3*S*)-Hmp]^2-nodupetide 2.

Table 1 ^{1}H-NMR and ^{13}C-NMR spectra data of [(2*S*,3*S*)-Hmp]^2-nodupetide compared to isolated nodupetide [3].

Assignment	**[(2*S*,3*S*)-Hmp]^2-nodupetide 2 ($CDCl_3$, ^{1}H-NMR 500 MHz, ^{13}C-NMR 125 MHz)**		**Nodupetide 1 (DMSO-$_{d6}$, ^{1}H-NMR 400 MHz, ^{13}C-NMR 100 MHz) [3]**	
	^{1}H δ in ppm (ΣH, m)	**^{13}C (ppm)**	**^{1}H δ in ppm (ΣH, m)**	**^{13}C (ppm)**
HMHA*				
CO				169.8
α-CH_2			2.25 (2H, dd)	37.7
β-CH			4.92 (1H, ddd)	75.3
γ-CH			1.58 (1H, m)	38.0
δ-CH_2			1.06 (2H, m)	24.4
σ-CH_3			0.83 (3H, t)	14.3
σ'-CH_3			0.84 (3H, d)	11.5
Ala^1				
CO		171.9		171.8
α-CH	3.69 (1H, bs)	49.1	4.19 (1H, q)	47.9
β-CH_3	1.44 (3H, d)	18.3	1.22 (3H, d)	17.4
D-Leu^2				
CO		171.2		171.2
α-CH	4.48 (1H, bs)	53.4	4.02 (1H, d)	51.8
β-CH_2	1.97 (2H, bs)	39.6	1.48 (2H, ddd)	38.6
γ-CH	1.41 (1H, d)	24.9	1.63 (1H, m)	24.1
δ-CH_3	0.90 (3H, d)	19.4	0.88 (3H, d)	19.0
δ'-CH_3	0.89 (3H, d)	23.0	0.80 (3H, d)	23.0
Val^3				
CO		172.5		171.7
α-CH	4.71 (1H, d)	59.8	4.03 (1H, m)	58.7
β-CH	1.83 - 1.85 (1H, m)	29.6	1.86 (1H, m)	29.5
γ-CH_3	0.93 (3H, d)	18.9	0.86 (3H, d)	18.9
γ'-CH_3	0.92 (3H, d)	17.5	0.84 (3H, d)	17.5
Gly^4				
CO		169.5		169.0
α-CH_2	4.13 (2H, bs)	43.5	4.07 (2H, dd)	41.9
(2*S*,3*S*)-Hmp				
CO		167.8		
α-CH	4.04 (1H, bs)	74.5		
β-CH	2.41 - 2.44 (1H, m)	37.5		
γ-CH_2	1.16-1.24 (2H, m)	25.4		

Assignment	**[(2*S*,3*S*)-Hmp]^{2}-nodupetide 2 (CDCl$_3$, ^{1}H-NMR 500 MHz, ^{13}C-NMR 125 MHz)**		**Nodupetide 1 (DMSO-$_{d6}$, ^{1}H-NMR 400 MHz, ^{13}C-NMR 100 MHz) [3]**	
	^{1}H δ in ppm (ΣH, m)	**^{13}C (ppm)**	**^{1}H δ in ppm (ΣH, m)**	**^{13}C (ppm)**
δ-CH$_3$	0.87 (3H, t)	14.2		
δ'-CH$_3$	0.91 (3H, d)	11.0		
Val^{6}				
CO		170.5		170.7
α-CH	4.71 (1H, d)	57.3	4.08 (1H, m)	57.7
β-CH	1.83 - 1.85 (1H, m)	29.5	2.07 (1H, m)	29.5
γ-CH$_3$	0.98 (3H, d)	18.6	0.85 (3H, d)	18.7
γ'-CH$_3$	1.00 (3H, d)	21.4	0.88 (3H, d)	20.9

Screening antimicrobial activity of [(2*S*,3*S*)-Hmp]^{2}-nodupetide (2)

The antimicrobial activity of [(2*S*,3*S*)-Hmp]^{2}-nodupetide and its linear was examined against 4 pathogenic microbes (**Table 2**). Based on growth inhibition data, [(2*S*,3*S*)-Hmp]^{2}-nodupetide and its linear were inactive against the 4 pathogenic microbes examined. MIC values > 1,000 ug/mL are considered inactive [10]. The substitution of HMHA with (2*S*,3*S*)-Hmp in nodupetide backbone probably alters the hydrophobicity due to differences in the position of their hydroxy groups and the length of their aliphatic carbon. It is plausible that the substitution of HMHA with (2*S*,3*S*)-Hmp in nodupetide was played a role in this dissimilarity in bioactivity. Increasing the hydrophobicity may hinder the access to membranes in prokaryotic cells [11].

Table 2 MIC values of [(2*S*,3*S*)-Hmp]^{2}-nodupetide and its linear peptide.

Peptides	**MIC (µg/mL)**			
	***Candida albican* (Fungi)**	***Pseudomonas aeruginosa* (Gram-negative)**	***Staphylococcus aureus* (Gram-positive)**	***Escherichia coli* (Gram-negative)**
Nodupetide 1	-	2.83	-	-
[(2*S*,3*S*)-Hmp]^{2}-nodupetide 2	> 1,000	> 1,000	> 1,000	> 1,000
Linear [(2*S*,3*S*)-Hmp]^{2}-nodupetide	> 1,000	> 1,000	> 1,000	> 1,000
Ciprofloxacin	0.156	0.005	0.078	15.625

Conclusions

[(2*S*,3*S*)-Hmp]^2-nodupetide was successfully synthesized using combination of solid and solution phase strategy and by placing the esterification in the last stage of elongation chain of peptide with overall yield 4.3 %. The antimicrobial activity of [(2*S*,3*S*)-Hmp]^2-nodupetide and its linear precursor was inactive, unlike their parent compound who has an active activity against *P. aeruginosa*. For further study, it may be beneficial to conduct computational study to identify prospective analogues with potential to exhibit antimicrobial activity.

Acknowledgements

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