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Synthesis of Cyclo-(D-Phe-D-Ala-Ile-Val-Leu-Gly), A Bacicyclin Analogue, Using A Combination of Solid- and Solution-Phase

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ABSTRACT

Bacicyclin is a cyclic peptide isolated from the bacterial culture of *Bacillus* sp. strain BC028 derived from blue mussel (*Mytilus edulis*) with a cyclo-(D-Phe-D-Ala-Ile-Val-Leu-Gly) sequence, which has antibacterial activity against *Enterococcus faecalis* and *Streptococcus aureus* with MIC values of 8 and 12 µM. The analogue was designed through the replacement of Ala residue to cationic residue, Lys, that is theoretically known to increase antibacterial activity to give an analogue of cyclo-(D-Phe-Lys-Ile-Val-Leu-Gly). The synthesis of the analogue was initiated by the synthesis of linear peptide using the solid phase method on 2-chlorotritylchloride (2-CTC) resin. All amino acid precursors were protected by fluorenylmethyloxycarbonyl (Fmoc) group, and a combination of coupling reagents 1-[bis(dimethyllamino) methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) were employed to facilitate all amide formations. The linear peptide was then removed from the resin using a trifluoroethanol (TFE):acetic acid (AcOH):dichloromethane (DCM) ratio (2:2:6) by maintaining the protecting group in the sidechain. Cyclization was carried out in solution phase by using benzotriazole-1-yloxytripyrrolidinophosphine hexafluorophosphate (PyBOP) as the cyclization agent and *N*,*N*-diisopropylmethylamine (DIPEA) as base. Deprotection of the *tertier*-butyl-oxylcarbonyl (Boc) protective group was carried out using trifluoroacetic acid (TFA):triisopropylamine (TIS): DCM with the ratio of 95:2.5:2.5. The synthetic product was successfully purified using semi-preparative RP-HPLC using acetonitrile (AcN) (0.1% TFA):water (0.1% TFA) as eluent with a combination of isocratic and gradient method starting from 20% until 80% AcN for 30 minutes. The target peak was found at tR = 18.443 minutes with the yield of 29,41%. Analysis of product purity using analytical RP-HPLC gives single peak at tR = 10.624 minutes. The synthesis results were characterized using HR-ToF-ESI-MS with $[M+H]^+$ = 658.4294 and [M+Na] = 680.4080

Keywords: antimicrobial peptide, bacicyclin, cyclohexapeptide, peptide cyclization, solid-phase synthesis.

INTRODUCTION

The increase in bacteria that are resistant to various types of antibiotic drugs has resulted in an increase in global health problems which has also increased mortality rates. In fact, diseases caused by infections by resistant bacteria are expected to cause millions of deaths by 2050, if no new antibiotics are approached [1].

In (2018), Tacconelli et al. conducted a study on the list of pathogenic bacteria, based on their resistant response to many antibiotics [2]. Gram-positive bacteria, *E. faecium*, which is resistant to the antibiotic

vancomycin and *S. aureus* bacteria, which are resistant to the antibiotic methicillin, have become a top priority in their treatment, because infections caused by these two bacteria are very fast and can infect many parts of the body, causing death.

One approach to find new antimicrobial agents is expected and one of them is antimicrobial peptides (AMP). AMP are widely produced by all organisms, where they have a broad spectrum of activity [1].

Antimicrobial peptide (AMP) is a peptide that has specific antimicrobial activity against various microorganisms such as Gram-positive bacteria, Gram-negative bacteria, fungi, and viruses [3]. In addition, this AMP peptide has broader benefits such as providing defense against pathogens, killing bacteria, stimulating the immune response system, regulating inflammation, being a biopesticide in plants and many other therapeutic benefits. An example of an antimicrobial peptide that has been widely used to treat blood infections and skin infections is daptomycin [4-5].

Sources of AMP also vary and have many benefits, one example is the blue clam mollusk *M. edulis* which has innate immunity against *E. faecalis* and *S. aureus* bacteria. Wiese and other researchers in (2018) found that the blue mussel (*M. edulis*) produces a source of antimicrobial peptides secreted by the body's defense system [6]. The antimicrobial peptide obtained was then isolated and its structure observed in the form of a cyclic peptide with six amino acids with a cyclo-(D-Phe-D-Ala-Ile-Val-Leu-Gly) sequence, which was then named bacicyclin. The isolated bacicyclin activity was tested by Wiese and colleagues against *E. faecalis* and *S. aureus* and obtained MIC values of 8.0 and 12.0 μM.

Cyclic peptides have many advantages such as high selectivity, efficiency and stability, and low molecular weight and toxicity when compared to linear peptides. This makes peptides with cyclic structures very good as drug candidates [7]. In addition, in a review of cyclic peptides as antibacterial compounds, cyclic peptides have the advantage of being able to bind more strongly to the bacterial membrane, so as to disrupt the stability of the bacterial membrane resulting in damage to the bacterial membrane, besides that cyclic peptides are also able to penetrate deeper into the bacterial membrane because it is able to form a β-sheet conformation [8]. Based on its more benefits, the development of this cyclic peptide continues to be intensively carried out, one of the approaches that can be carried out is analog synthesis and also the structure activity relationship approach to cyclic peptides.

In (2021), Chen et al. have succeeded in synthesizing six analogues of the cyclic bacicyclin hexapeptide by changing the configuration of the constituent amino acids of bacicyclin, and also by replacing one of the amino acids used with a non-natural amino acid [9]. The results of the synthesis of this analog succeeded in increasing the antitumor activity of the bacicyclin compound, but the resulting antimicrobial activity gave moderate results, so further research on bacicyclin analogues is needed to obtain other analogues with better antimicrobial activity. Therefore, new research is needed regarding the synthesis of bacicyclin analogues, one of which is an analogue with cyclo-(D-Phe-Lys-Ile-Val-Leu-Gly) sequences which will be synthesized using a combination of solid and solution phase peptide synthesis methods. In this bacicyclin analog, the amino acid residue Ala was replaced with the amino acid Lys because theoretically it was able to increase its antibacterial activity [10-11]. The combination of solid and solution phase peptide synthesis was chosen because it has been successfully used to synthesize other cyclic peptides such as c-PLAI [12-15].

Solid phase peptide synthesis is a peptide synthesis method that uses the help of a solid support (resin) attached to the first amino acid [16]. This solid phase synthesis strategy is used because it only requires a short time, and involves less purification process [12]. The resin used is 2-CTC resin because it can reduce racemization and eliminate the side reaction of diketopiperazine formation [17]. HATU and HOAt coupling reagents are also used in the synthesis of solid phase peptides which function to form active esters so that coupling of amino acids can occur. The Lys amino acid which has an amine functional group on its side chain needs to be protected by the presence of a Boc side chain protecting group, so that the coupling process is not disturbed. This solid phase synthesis was carried out in order to obtain linear peptides. Linear peptide cyclization was carried out using the solution phase peptide synthesis method with the Gly cyclization point as the C terminal because it is a β-Turn Inducer and because it has no side groups it can avoid epimerization. Meanwhile, D-Phe was chosen as the N terminal because it has a D conformation which also includes a β-Turn Inducer which can help the cyclization process.

Based on this background, it is expected that this analogue of bacicyclin can be an alternative antibacterial compound that can be synthesized to fight infections from *Enterococcus faecalis* and *Staphylococcus aureus* bacteria.

EXPERIMENTAL SECTION

Material and Instrumental

The amino acids used in this study were the amino acids L-Gly, L-Leu, L-Val, L-Ile, L-Lys(Boc), and D-Phe whose N-terminus was protected by the Fmoc group (Fmoc-AA- OH). 2-Chlorotrithyl Chloride (2-CTC) resin, *N*,*N*-dimethylformamide (DMF) and Dichloromethane (DCM) solvent, *N*,*N*-diisopropylethylamine (DIPEA) base, piperidine, methanol (MeOH), a mixture of 2% acetaldehyde solution in DMF and 2% p-chloranyl solution in DMF for the chloranyl test, coupling reagent 1-[Bis(dimethyllamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), 1-Hydroxy-7-azabenzotriazole (HOAt), and benzotriazole-1-yloxytripyrrolidinophosphine hexafluorophosphate (PyBOP), Scavenger triisopropylsilane (TIPS), trifluoroacetic acid solution (TFA), trifluoroethanol (TFE), and glacial acetic acid (AcOH). The extraction of cyclized peptides was carried out using 100% NaCl solution in H_2O.

The instrument used is Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) brand Waters type Perkins 2965 used for purification of linear peptides and cyclic peptides, High Resolution Mass Spectrometer Time of Flight instrument with Electron Spray Ionization (ESI) system (HR-ToF -ESI-MS) brand Waters is used to test product purity, and the instrument UV-Vis Spectrophotometer brand Perkin Elmer is used for LR testing when synthesizing linear peptides.

Methods

Synthesis of linear bacicyclin analogue (FKIVLG)

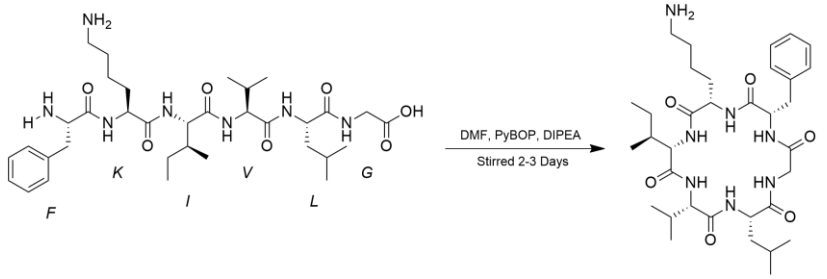
CTC resin (0.375 mmol) was added to 5 mL DCM and then shaken for 30 minutes (twice). The resin was added to the first amino acid solution, namely Fmoc-L-Gly-OH (1eq, dissolved in 5mL DCM and added 2eq DIPEA), homogenized, then shaken for 24 hours. The resin was dried and its loading resin value was measured. Resin was then capped using methanol:DCM:DIPEA (15:80:05 v/v). The Fmoc group was removed using 4 mL of a 20% solution of piperidine in DMF (duplo) and the reaction was monitored by a chloranyl test.

The second amino acid (3 eq.) solution in DMF with the addition of 3eq HATU, 3eq HOAt, and 6eq DIPEA was added to the resin-Fmoc-Gly-NH_2. The coupling process of the amino acids was carried out by shaking the resin for 24 hours and the chloranyl test was carried out to monitor each coupling and Fmoc deprotection. The third to sixth amino acids were added sequentially using 3eq amino acids, 3eq HATU, 3eq HOAt, and 6eq DIPEA with a repetitive protocol (Fmoc deprotection and coupling) untuk resin-hexapeptidyl resin was obtained.

Resin cleavage was carried out using an acid solution of TFE:AcOH:DCM (2:2:6 v/v) for 2 hours. The linear peptide filtrate was then dried and characterized using HR-ToF-ESI-MS with a bacicyclin analog linear peptide molecular mass of 775.48 g/mol.

HR-ToF-ESI-MS results showed a peak spectrum of *m/z* 776.4934, which is $[M+H]^+$ of the bacicyclin analog linear peptide.

Cyclization of linear bacicyclin analogue (FKIVLG)



16.1 mg of the linear bacicyclin analogue was dissolved in DMF to make a solution concentration of 2.09 mM (9.90 mL DMF), then PyBOP (1.14 eq), base DIPEA (1.65 eq), and NaCl solution in H_2O with a concentration of 1mM were added. The mixture was stirred for 2-3 days.

The reaction mixture was then extracted using ethyl acetate and 100% NaCl in H_2O thrice. The organic phase was concentrated and a protected bacicylin analogue crude was dried and characterized using HR-ToF-ESI-MS. HR-ToF-ESI-MS results showed a peak spectrum of *m/z* 780.7520 which is $[M+Na]^+$ of protected bacicylin analog cyclic peptide ion.

The protected bacicylin analogue was deprotected for its Boc protective group using a TFA:TIS:DCM acid solution with a ratio (95:2.5:2.5 v/v) and the reaction mixture was shaken for 2 hours, the product was dried and confirmed again using HR-ToF- ESI-MS. HR-ToF-ESI-MS results show a peak spectrum *m/z* 658.4276 which indicates $[M+H]^+$ of the bacicylin analog cyclic peptide ion.

Bacicyclin analogue purification

The cyclic peptide analogue of bacicylin was purified using a semi-preparative RP-HPLC instrument with a non-polar C-18 (ODS) column with a particle size of 300 Å, a pore size of 5 µm, and a length of 250 x 10mm. The prepared peptide solution was injected as much as 1000 µL with the mobile phase of 0.1% TFA in AcN and 0.1% TFA in H_2O using the eluent gradient method. The flow rate used was 2.00 mL/minute and PDA detector with a wavelength of 210, 240, 254 nm were applied. The peptide was dissolved in 100% methanol until homogeneous and injected into the instrument. The purification results show that the chromatogram with the target peak appears at a retention time of 18.443 minutes with an acetonitrile concentration of 70%. The eluent fraction was dried with the help of freeze dry and a pure bacicylin analog cyclic peptide was obtained.

Cyclic peptide analog of bacicylin was confirmed again with HR-ToF-ESI-MS and found the peak of the target spectrum with *m/z* 658.4294 indicating $[M+H]^+$ and with *m/z* 680.4080 indicating $[M+Na]^+$ from the peptide ion cyclic analog bacicyclin.

RESULTS AND DISCUSSION

Synthesis and characterization of linear bacicyclin analogue (FKIVLG)

Synthesis of linear peptide of bacicylin analogue with FKIVLG sequences was carried out on 2-CTC resin with a Fmoc synthetic strategy and the use of HATU and HOAt as coupling reagents. 2-CTC resin needs to be swollen first with DCM solvent so that the first amino acid, namely Fmoc-Gly-OH, binds maximally. The 2-CTC resin that has been developed (swollen) was added by the first amino acid solution, Fmoc-Gly-OH in DCM in the presence of DIPEA. The 2-CTC resin that has been added by the amino acid solution will release the Cl- group because it acts as a good leaving group and forms a tertiary resin carbocation, which has a positive charge. DIPEA, which acts as a Bronsted-Lowry base will deprotonate protons in the carboxylic groups of the first amino acid, so that a nucleophile (O-) is obtained from the amino acid glycine, which will attack the tertiary carbocation in 2-CTC resin. The first amino acid binding reaction with the 2-CTC resin is a unimolecular nucleophilic substitution reaction (S_N1), through this reaction an ester bond is formed between the amino acid and the 2-CTC resin (Figure 1).

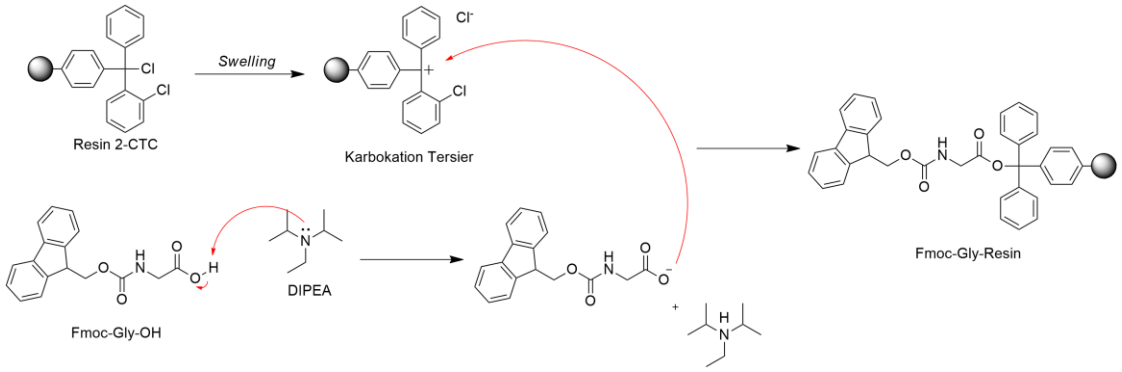


Figure 1. Mechanism of binding of the first amino acid Fmoc-Gly-OH to 2-CTC resin with the help of base DIPEA.

The resin that has bound the first amino acid is then measured for its resin loading value to find how many Gly amino acids are bound to the resin. The amount of Gly amino acid that binds to the resin is directly proportional to the amount of dibenzofulvene (DBF) produced as a result of the deprotection reaction. The amount of DBF will be read by the Uv-Vis spectrophotometer instrument at a wavelength of 290 nm because it has a chromophore group derived from benzene.

The resin loading calculations gave a resin loading value of 0.6687 mmol/g, which is in the range of good resin loading values. The active site was then capped to prevent other amino acids from sticking to the resin using a combination DCM:MeOH:DIPEA solution with a ratio (80:15:5). The added methanol will act as a nucleophile which will attack the unreacted active site of the tertiary carbocation that has been formed, then the DIPEA base will act as a deprotonating agent in methanol, which binds to the resin so that the final product is formed in the form of a less reactive ether.

The next step is to remove the Fmoc protective group on the resin-Gly-Fmoc to give free amino group. The piperidine base acts as a Bronsted-Lowry base capable of deprotonating a proton from the Fmoc group, which will eventually give DBF and CO_2 gas (Figure 2).

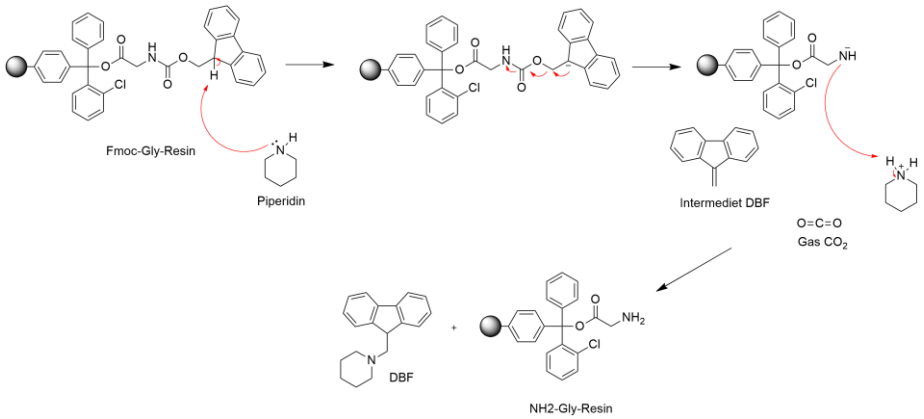


Figure 2. Mechanism of deprotection reaction for Fmoc groups using 20% piperidine solution in DMF.

The success of this deprotection procedure was observed by the chloranyl test, which was carried out after the deprotection was complete. The chloranyl test was carried out to test for the presence of free amino group in the sample, where if there is free amino group, which indicates a successful deprotection procedure, the resin will change color from yellow to green/blue/red indicating a positive result. The results of this chloranyl test showed a change in color, so the synthesis procedure was continued to the amino acid coupling.

Amino acid coupling was initiated by adding a coupling reagent mixture containing a second amino acid, Fmoc-Leu-OH (3 equivalents) and coupling reagents HATU (3 equivalents) and HOAt (3 equivalents). The solid mixture is dissolved in DMF and then 6 equivalents of DIPEA base is added to the solution. Amino acid coupling occurs heterogeneously between amino acid solution and also the resin that bind the first amino acid, so that the coupling reagent equivalents and also the added base need to be in excess. Initially, the coupling reagent and additives, HATU and HOAt, will activate the carboxylic group of the second amino acid, with the help of basic DIPEA so that an active ester intermediate is formed. HOAt, which is a partner additive from HATU will help the process of forming active ester compounds and directing the reaction to the formation of active ester. The addition of HOAt can also reduce the side reactions of racemate formation (Figure 3). The ester group formed has better leaving group properties compared to the OH group in the previous carboxylic acid. The combination of addition and elimination reactions results in a new peptide bond between the first amino acid Gly and the second amino acid Leu.

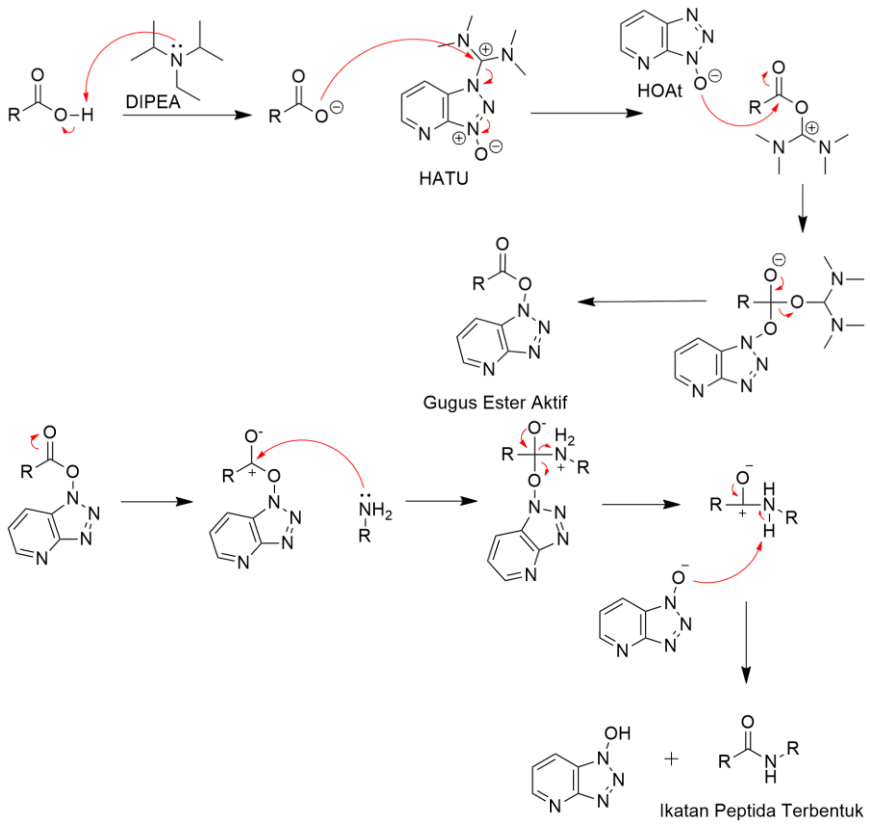


Figure 3. The reaction mechanism of coupling reaction.

The success of the coupling reaction was observed using a qualitative chloranyl test where if the amino acid coupling reaction was successful then the previously existing free amine group would be lost because it had formed a peptide bond with the second amino acid.

Fmoc deprotection and coupling of amino acids were carried out repeatedly under the same reaction conditions until the desired linear bacicyclin analog was obtained, with the FKIVLG sequence (Figure 4).

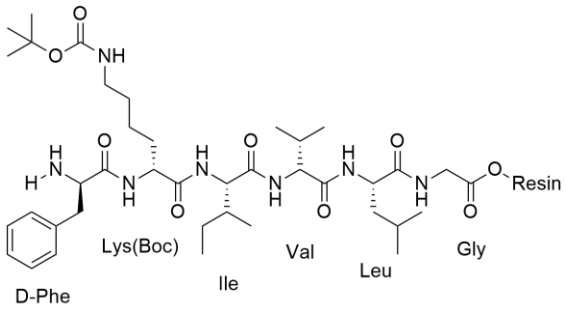


Figure 4. Structure of FKIVLG.

The linear peptide of bacicyclin analog (FKIVLG), which has been successfully synthesized was then released from 2-CTC resin using a combination of trifluoroethanol (TFE) solution: acetic acid (AcOH): and DCM solvent ratio (1:1:3). The acid solutions used in this procedure are TFE and AcOH, which are weak acid solutions. TFE has a pKa of 12.46 and AcOH has a pKa of 4.76, so a combination of the two is needed to be able to release the resin from the peptide chain that has been formed. The acid solution used is mild because the desired product is a linear peptide crude, which still has the Boc protection group on the Lys residue but is detached from the 2-CTC resin.

The filtrate obtained was then concentrated using a rotary evaporator with the help of DCM precipitation agents, n-hexane, ethyl acetate, and methanol to remove the acid solution used. The crude of linear bacicyclin analog is obtained as white solid.

Crude of linear bacicyclin analogue was characterized using HR-ToF-ESI-MS to confirm that the compound has the same molecular weight as the synthetic product (linear bacicyclin analogue = 775.48 g/mol). The mass spectra (Figure 5) shows one dominant peak (green box) with *m/z* 776.4934 which is $[M+H]^+$ of the linear bacicyclin analogue and one minor peak (red box) with *m/z* 676.4725, which is the $[M]^+$ of the deletion compound ion peak caused by the deletion of the Val residue. The deletion of the Val amino acid occurs as a result of failure coupling between resin-Gly-Leu-NH_2 and Fmoc-Val-COOH.

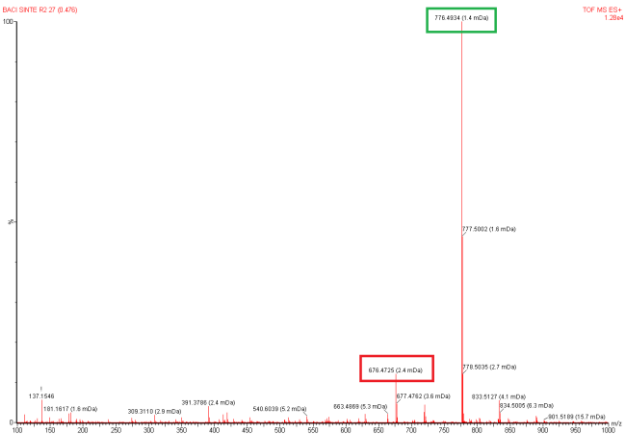


Figure 5. HR-TOF-MS spectra of crude of linear bacicyclin analogue

The linear bacicyclin analogue, which has been confirmed by the HR-ToF-ESI-MS was then observed for its purity using analytical RP-HPLC with C-18 column (particle size of 300.00 Å, pore size of 5.00 µm, and a length of 150 x 4.6mm) connected to a PDA detector at wavelengths of 210, 240, and 254 nm. The flow rate used was 1mL/minute with 0.1% TFA in AcN and 0.1% TFA in H_2O using a 20 – 80% AcN concentration gradient method for 30 minutes. The chromatogram shows several peaks as can be seen in Figure 6.

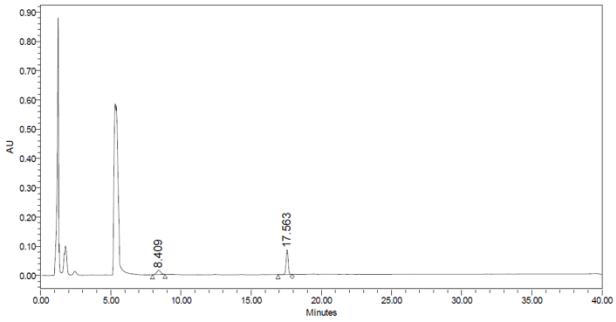


Figure 6. Chromatogram of analytical HPLC of crude of linear bacicyclin analogue.

Peptide cyclization was carried out in solution phase (Figure 7). The cyclization was carried out in three different conditions. The first condition of cyclization was carried out using only HATU coupling reagent with DMF solvent and DIPEA base. The first condition cyclization was carried out for 2 days and when confirmed by HR-ToF-ESI-MS, cyclic peptides were not found. The second condition was carried out using a combination of PyBOP and HOAt coupling reagent, with DCM solvent and DIPEA base, the cyclization results of this second condition also showed a negative result, because the cyclic product was not formed after being confirmed using HR-ToF-ESI-MS. The third cyclization condition uses PyBOP, NaCl and DIPEA and fortunately it successfully provides the desired cyclic product. Sodium chloride salt is used, to bring the C-terminal and N-terminal ends closer together by forming ionic bonds with the carbonyl groups in the peptide chain, so that when the two ends of the peptide chain are close, coupling of amino acids becomes easy.

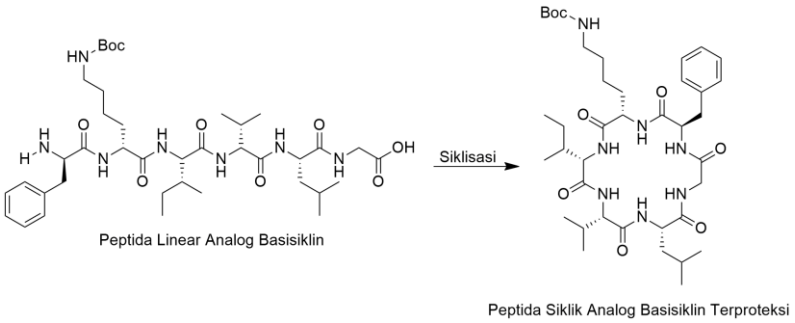


Figure 7. Cyclization of linear precursor of bacicyclin analog.

The peptide solution was extracted using two solvents that have significant polarity differences (liquid-liquid extraction). In this extraction process used ethyl acetate solvent, which is non-polar and 100% NaCl solution in H_2O which is polar. The cyclic peptide that is formed will dissolve in ethyl acetate which is non-polar according to the like-dissolve-like principle. Meanwhile, other impurities such as linear peptide precursors that fail to react, NaCl salts, PyBOP coupling reagents that do not react will dissolve in 100% NaCl solution in H_2O and precipitate into a white solid through the salting out process. The organic phase obtained was collected and concentrated using a rotary evaporator. The cyclic peptide was obtained as a yellowish crude.

The cyclic formation was confirmed using the HR-ToF-ESI-MS (molecular mass of cyclic peptide analog = 757.47 g/mol (Figure 8). The mass spectra showed *m/z* 780.7520 which is $[M+Na]^+$ of the cyclic bacicyclin analog, and another peak at m/z 775, 7876 which is the [M]+ of the linear precursor of bacicyclin analog. The MS spectrum obtained showed that the peak intensity of the bacicyclin analog was relatively shorter compared to the impurities, but it can be concluded that the cyclization method used the PyBOP was successful but with low yield.

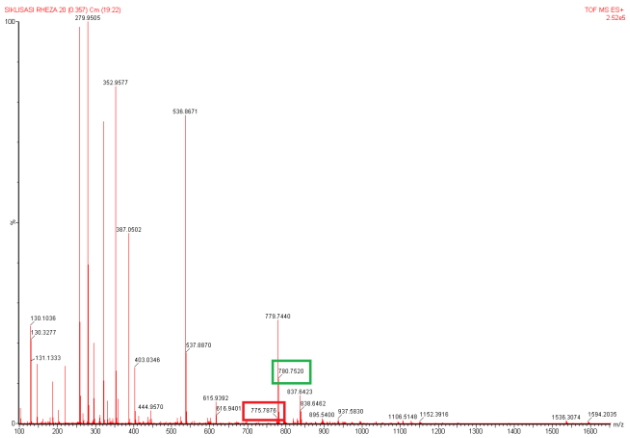


Figure 8. HR-TOF-MS spectra of crude of Boc-protected bacicyclin analogue.

The crude of cyclic bacicyclin analog was then added by a mixture of trifluoroacetic acid (TFA): triisopropylsilane (TIS): DCM (95:2.5:2.5). This step was carried out to deprotect the Boc protecting group from the crude cyclic bacicyclin analog (Figure 9).

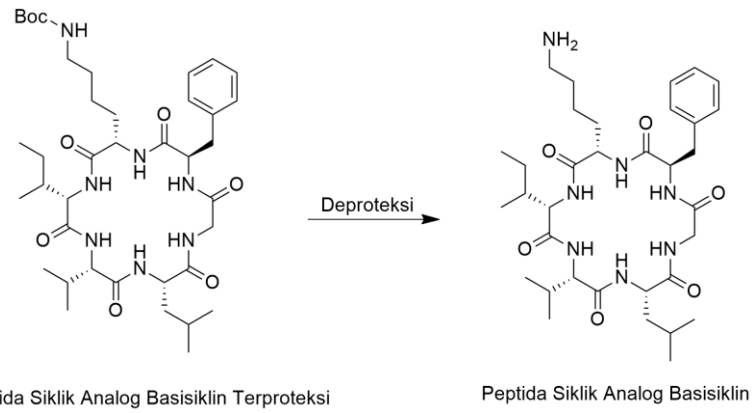


Figure 9. Deprotection in protected bacicylin analogue cyclic peptides.

Deprotection of the Boc protective group resulted yellowish oil crude. Deprotection result was confirmed using HR-ToF-ESI-MS (molecular mass of cyclic bacicyclin analog = 657.42 g/mol). The HR-TOF-MS spectrum (Figure 10) shows *m/z* 658.4276, which is $[M+H]^+$ of the desired bacicyclin analog It can be concluded that the deprotection protocol was successfully carried out. Bacicyclin analog was purified using semi-preparative RP-HPLC in order to obtain pure bacicyclin analog.

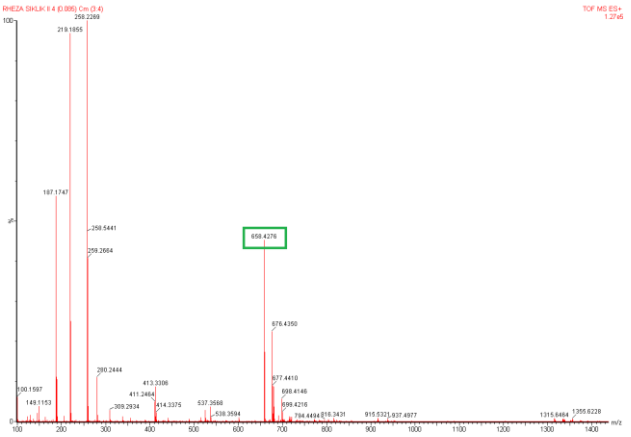


Figure 10. HR-TOF-MS spectra of crude of bacicyclin analogue.

Cyclic peptide analogues were purified using a semi-preparative RP-HPLC instrument with C-18 (ODS) column with a particle size of 300 Å, a pore size of 5 µm, and a length of 250 x 10mm. The peptide solution was injected for approximately 1000 µL with the mobile phase of 0.1% TFA in AcN and 0.1% TFA in H_2O using the gradient and isocratic combination method for 30 minutes. The flow rate used was 2.00 mL/minute and was observed with the help of a PDA detector with a wavelength of 210, 240, 254 nm. Fraction S2 fraction (rt = 18.443 minutes) showed the presence of *m/z* 658.4294, which is $[M+H]^+$ from the bacicyclin analogue and *m/z* 680.4080, which is also $[M+Na]^+$ from the cyclic peptide bacicyclin analog.

Bacicylin analog was analyzed for its purity using analytical RP-HPLC instrument with C-18 column with a particle size of 300.00 Å, a pore size of 5.00 µm, and a length of 150 x 4.6mm, which is connected to a PDA detector which will read at a wavelength of 210, 240, and 254 nm. The flow rate used was 1 mL/minute with 0.1% TFA in AcN and 0.1% TFA in H_2O using a 20 – 80% AcN concentration gradient method for 30 minutes. The chromatogram (Figure 11) show a single peak at a retention time of 10.624, which was suspected to be a bacicyclin analog.

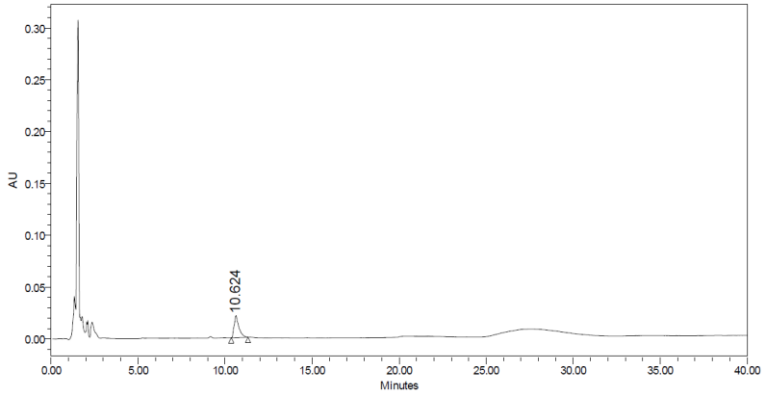


Figure 11. Chromatogram of analytical RP-HPLC of purified cyclic bacicyclin analog.

CONCLUSION

Cyclic baciicyclin analogue (c-FKIVLG) was successfully synthesized using a combination of solid and solution phase peptide synthesis methods with a yield of 29.81%, which was confirmed by the HR-ToF-ESI-MS spectrum.

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REFERENCES

1) Torres, M.D.T., Sothiselvam, S., Lu, T.K., & de la Fuente-Nunez, C. (2019) Peptide Design Principles for Antimicrobial Applications. *Journal of Molecular Biology*. **431**(18), 3547–3567
2) Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E.M., Houchens, C.R., Grayson, M.L., Hansen, P., Singh, N., Theuretzbacher, U., Magrini, N., Aboderin, A.O., Al-Abri, S.S., Awang Jalil, N., Benzonana, N., Bhattacharya, S., Brink, A.J., Burkert, F.R., Cars, O., Cornaglia, G., Dyar, O.J., Friedrich, A.W., Gales, A.C., Gandra, S., Giske, C.G., Goff, D.A., Goossens, H., Gottlieb, T., Guzman Blanco, M., Hryniewicz, W., Kattula, D., Jinks, T., Kanj, S.S., Kerr, L., Kieny, M.P., Kim, Y.S., Kozlov, R.S., Labarca, J., Laxminarayan, R., Leder, K., Leibovici, L., Levy-Hara, G., Littman, J., Malhotra-Kumar, S., Manchanda, V., Moja, L., Ndoye, B., Pan, A., Paterson, D.L., Paul, M., Qiu, H., Ramon-Pardo, P., Rodríguez-Baño, J., Sanguinetti, M., Sengupta, S., Sharland, M., Si-Mehand, M., Silver, L.L., Song, W., Steinbakk, M., Thomsen, J., Thwaites, G.E., van der Meer, J.W., Van Kinh, N., Vega, S., Villegas, M.V., Wechsler-Fördös, A., Wertheim, H.F.L., Wesangula, E., Woodford, N., Yilmaz, F.O., & Zorzet, A. (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*. **18**(3), 318–327.
3) Zasloff, M. (2002) Antimicrobial peptides of multicellular organisms. *Nature*. **415**, 389–395.
4) Magana, M., Pushpanathan, M., Santos, A.L., Leanse, L., Fernandez, M., Ioannidis, A., Giulianotti, M.A., Apidianakis, Y., Bradfute, S., Ferguson, A.L., Cherkasov, A., Seleem, M.N., Pinilla, C., de la Fuente-Nunez, C., Lazaridis, T., Dai, T., Houghten, R.A., Hancock, R.E.W., & Tegos, G.P. (2020) The value of antimicrobial peptides in the age of resistance. *The Lancet Infectious Diseases*. **20**(9), e216–e230.
5) Moreira, R., Wolfe, J., & Taylor, S.D. (2021) A high-yielding solid-phase total synthesis of daptomycin using a Fmoc stable kynurenine synthon. . **19**(April), 3027–3264.
6) Wiese, J., Abdelmohsen, U.R., Motiei, A., Humeida, U.H., & Imhoff, J.F. (2018) Bacicyclin, a new antibacterial cyclic hexapeptide from Bacillus sp. strain BC028 isolated from Mytilus edulis. *Bioorganic and Medicinal Chemistry Letters*. **28**(4), 558–561.
7) Abdel Monaim, S.A.H., Acosta, G.A., Royo, M., El-Faham, A., de la Torre, B.G., & Albericio, F. (2018) Solid-phase synthesis of homodetic cyclic peptides from Fmoc-MeDbz-resin. *Tetrahedron Letters*. **59**(18), 1779–1782.

8) Mika, J.T., Moiset, G., Cirac, A.D., Feliu, L., Bardají, E., Planas, M., Sengupta, D., Marrink, S.J., & Poolman, B. (2011) Structural basis for the enhanced activity of cyclic antimicrobial peptides: The case of BPC194. *Biochimica et Biophysica Acta - Biomembranes*. **1808**(9), 2197–2205.
9) Chen, Q., Wu, M. hao, Chang, Q., & Zhao, X. (2021b) Total synthesis and modification of Bacicyclin (1), a new marine antibacterial cyclic hexapeptide. *Tetrahedron Letters*. **63**(xxxx), 152705.
10) Monera, O.D., Sereda, T.J., Zhou, N.E., Kay, C.M., & Hodges, R.S. (1995) Relationship of sidechain hydrophobicity and α-helical propensity on the stability of the single-stranded amphipathic α-helix. *Journal of Peptide Science*. **1**(5), 319–329.
11) Sereda, T.J., Mant, C.T., Sönnichsen, F.D., & Hodges, R.S. (1994) Reversed-phase chromatography of synthetic amphipathic α-helical peptides as a model for ligand/receptor interactions Effect of changing hydrophobic environment on the relative hydrophilicity/hydrophobicity of amino acid side-chains. *Journal of Chromatography A*. **676**(1), 139–153.
12) Napitupulu, O.I., Sumiarsa, D., Subroto, T., Nurlelasari, Harneti, D., Supratman, U., & Maharani, R. (2019) Synthesis of cyclo-PLAI using a combination of solid- and solution-phase methods. *Synthetic Communications*. **49**(2), 308–315.
13) Maharani, R., Napitupulu, O.I., Dirgantara, J.M., Hidayat, A.T., Sumiarsa, D., Harneti, D., Nurlelasari, Supratman, U., & Fukase, K. (2021) Synthesis of cyclotetrapeptide analogues of c-PLAI and evaluation of their antimicrobial properties. *Royal Society Open Science*. **8**(3).
14) Rahim, A., Hidayat, A.T., Nurlelasari, Harneti, D., Supratman, U., & Maharani, R. (2020) A total synthesis of cyclodepsipeptide [Leu]6-aureobasidin k using combination of solid-and solution-phase. *Current Chemistry Letters*. **9**(2), 97–104.
15) Rahmadani, A., Masruhim, M.A., Rijai, L., Hidayat, A.T., Supratman, U., & Maharani, R. (2021) Total synthesis of cyclohexadepsipeptides exumolides A and B. *Tetrahedron*. **83**, 131987.
16) Merrifield, R.B. (1963) Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *Journal of the American Chemical Society*. **85**(14), 2149–2154.
17) Lee, Tae Kyung, Ryoo, S.J., & Lee, Y.S. (2007) A new method for the preparation of 2-chlorotrityl resin and its application to solid-phase peptide synthesis. *Tetrahedron Letters*. **48**(3), 389–391.