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A total synthesis of cyclodepsipeptide [Leu]^6-aureobasidin K using combination of solid- and solution-phase

Andi Rahim[a], Ace Tatang Hidayat[a,b], Nurlelasari[a], Desi Harneti[a], Unang Supratman[a,b] and Rani Maharani[a,b,*]

[a]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, West Java, Indonesia*
[b]*Laboratorium Sentral, Universitas Padjadjaran, Jatinangor, West Java, Indonesia*

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[Leu]^6-aureobasidin K **1** is an analog of AbK, in which *a*Ile residue at the sixth position of AbK is replaced by Leu. [Leu]^6-AbK **1** was successfully synthesized using a combination of solid- and solution-phase that has a difference with the previous method applied for the synthesis of other aureobasidin analogues, [2*S*,3*S*-Hmp]-AbL **2**. The linear precursor was prepared by using solid-phase method with Fmoc strategy, in which an ester bond was constructed on the resin. The cyclic product was obtained from a solution-phase reaction of the linear precursor using HATU reagent. The crude of the cyclic peptide was purified using a column chromatography technique to obtain [Leu]^6-AbK **1** with a percent yield of 5.31% that is higher compared to previous similar method for synthesis of [2*S*,3*S*-Hmp]-AbL **2**. The purity level of cyclic product was analyzed using analytical RP-HPLC (t_R = 6.49 min) and analysis of structure using ^{1}H-NMR.

1. Introduction

Aureobasidins are a class of cyclodepsipeptides, isolated from black yeast, *Aureobasidium pullulans* R106 with prominent antifungal properties. There are over 30 members of aureobasidin derivatives have been successfully isolated and characterized. Most of aureobasidins possess three or four of *N*-methylated amino acids and one hydroxy acid. The structures are cyclic with one ester bond (depside).[1–3]

To date, there are only four papers describing total synthesis of aureobasidin, where three of the synthesis were carried out in solution phase[4–6] and one synthesis was undertaken using a combination of solid- and solution-phase method[7]. The later method, applied for the synthesis [2*S*,3*S*-Hmp]-aureobasidin L **2** (**Fig. 1**), was found to be faster than the previous three methods, although the yield of synthesis was still low. In the synthesis of [2*S*,3*S*-Hmp]-aureobasidin L **2**, Maharani *et al.*[7] synthesized an ester bond of [2*S*,3*S*-Hmp]-AbL **2** in solution-phase as a depsidipeptide substructure of Fmoc-

* Corresponding author. Tel.: +6281290184903
E-mail address: r.maharani@unpad.ac.id (R. Maharani)

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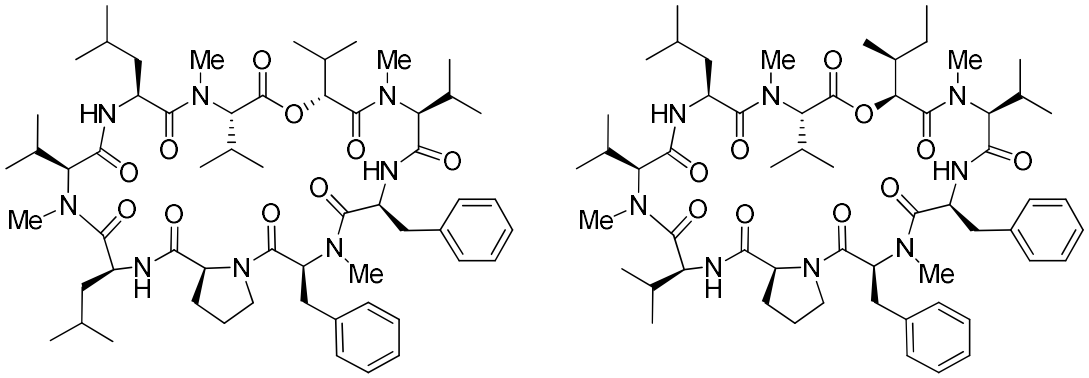
MeVal-O-Hmp-OH **3**. This depsidipeptide was then attached onto the peptidyl resin to construct the linear peptide with an overall yield of 1.89%.

In this current report, [Leu]^6-aureobasidin K **1** was chosen as target of synthesis. The strategy of the synthesis was similar to the strategy used by Maharani *et al*.[7] which is through a combination of solid- and solution-phase methods. However, a different approach of the synthesis was applied. In the total synthesis of the linear peptide of [Leu]^6-AbK **1**, an ester bond formation was undertaken on the resin[8–11]. Gimeno *et al.*[11] showed that an ester bond formation on resin using DIC/DMAP can be facilitated easily to give high yield of the product. Due to this, it was planned to attached the hydroxy acid, D-Hiv **5**, onto resin directly using the method used by Gimeno *et al.*[11]. The hydroxy acid was synthesized by using a protocol reported by Muller *et al*.[12] and protected by Fmoc using a procedure of Kuisle *et al.*[8]. A position between proline as *N*-terminus and methyl phenylalanine as *C*-terminus was selected as cyclization site. Proline is selected as *N*-terminus since it can support the cyclization process by β-turn inducer as its features.[13] This method was expected to increase yield of the synthesis of [Leu]^6-AbK **1**.

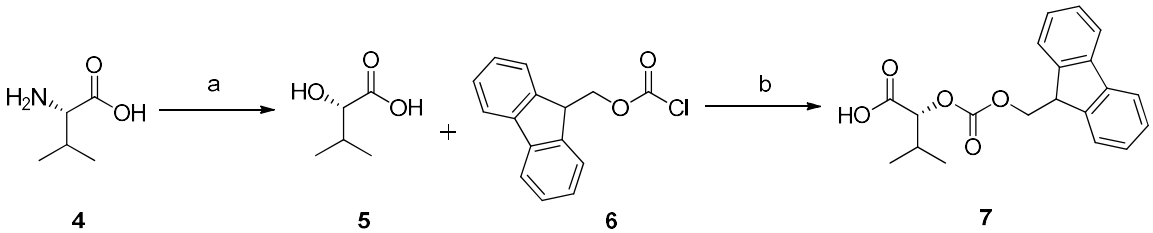


[Leu]^6-AbK **1** [2*S*,3*S*-Hmp]-AbL **2**

Fig. 1. Structures of [Leu]^6-AbK **1** and [2*S*,3*S*-Hmp]-AbL **2**

2. Results and Discussion

The initial step for the total synthesis of [Leu]^6-AbK **1** is the preparation of Fmoc-D-Hiv-OH **7**. Fmoc-D-Hiv-OH **7** was prepared through a two-step reaction involving the conversion of D-valine **4** to (*R*)-2-hydroxy-3-methylbutanoic acid or D-hydroxyisovaleric acid (D-Hiv) **5** through a diazotization reaction and then was continued with the protection of D-Hiv **5** by Fmoc-Cl **6** to make (*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)oxy)-3-methylbutanoic acid or Fmoc-D-Hiv-OH **7** (**Scheme 1**).



4 **5** **6** **7**

Scheme 1. Synthesis of (*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)oxy)-3-methylbutanoic acid. Reagents and conditions: (a) $NaNO_2$ in water, 1 N of H_2SO_4, 0°C for 3 h, and then rt for overnight, (88.2%). (b) Pyridine, Fmoc-Cl, THF, 0°C for 3 h, and then rt for overnight, cyrstallized in toluene, (51%).

The linear nonadepsipeptide of [Leu]^6-AbK **17** was prepared through solid-phase method (**Scheme 2**). In the synthesis of the linear nonadepsipeptide, Fmoc-L-MePhe was attached on resin as the first amino acid. This step took place in dichloromethane with the presence of DIPEA as the base for overnight. After that, 20% piperidine in DMF was employed for the deprotection of Fmoc-group to give a free amino group that was ready to be coupled with second amino acid, Fmoc-L-Phe.

The coupling with Fmoc-L-Phe was facilitated with HATU/HOAt as the coupling reagent and DIPEA as the base in DMF. The presence of highly *N*-methylated amino acids on the resin such as Fmoc-L-MePhe and Fmoc-L-MeVal has made the application of a double coupling protocol inevitable to result in a complete reaction. Each protocol employed a proportion of amino acid/HATU/HOAt/DIPEA of 3:3:3:6 equivalent that was conducted 1x4 h or 2x4 h.[14] The synthetic issue was found during the attachment of Fmoc-D-Hiv as the fourth amino acid onto the tripeptidyl **10** with Fmoc-L-MeVal as *N*-terminus to result in tetrapeptide-OH **11**. To make a success coupling, a four time-coupling protocol was required. This can be explained due to the presence of a β-branch structure from both residues involved in the amide bond formation, that has made the coupling difficult.

In the following step, the attachment of Fmoc-L-MeVal as the fifth residue involved the ester bond formation to the linear tetrapeptide-OH **11** on the resin. This step employed DIC/DMAP as a cocktail of coupling reagent at 45°C over 3x5 h, following a protocol by Gimmeno *et al.*[11] for the synthesis of pipecolidepsin A. Gimmeno *et al.*[11] mentioned that the protocol could give very high performance in the ester bond formation. Unfortunately, the attachment of the Fmoc-L-MeVal to the linear tetrapeptide-OH **11** on the resin showed incomplete coupling after a three-time attempt. The unsuccessful process was analyzed by HR-TOF-MS, which showed the presence of molecular ion peaks of both the uncoupled linear tetrapeptide-OH **11** and the desired linear of pentadepsipeptide **12** on the resin. The presence of β-branch structures from both sides of the residues was the main cause of the incomplete ester bond formation. The peptidyl resin with a mixture of two products (**11** and **12**) was continued for the following step.

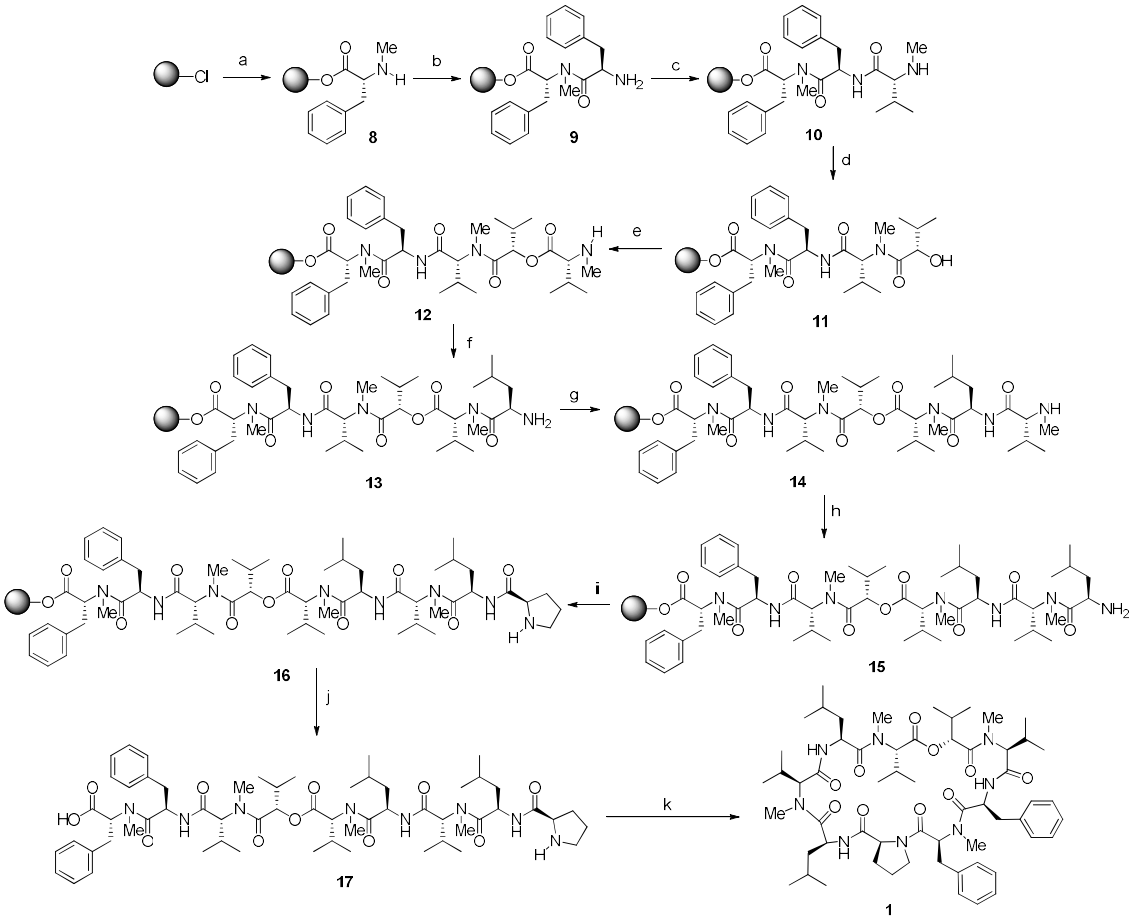
In the next step, Fmoc-L-Leu as the sixth amino acid was attached to pentadepsipeptide **12** on the resin by the employment of a cocktail of HATU/HOAT/DIPEA in DMF to form hexadepsipeptidyl resin **13**. The cocktail of 20% piperidine in DMF was used as a cocktail for the deprotection of Fmoc-group of **13** to give linear hexadepsipeptide-NH_2 **13**. Then, HR-TOF-MS was applied for the analysis of **13**. The HR-TOF-MS analysis showed the absence of the linear **13** and also peptide **12** as the precursor. However, the MS analysis only showed the presence of tetrapeptide-OH **11**. It seems likely that diketopiperazine (DKP) formation might take place during the Fmoc deprotection using piperidine. A similar finding has been reported by Maharani *et al*.[7] in the total synthesis of [2*S*,3*S*-Hmp] AbL **2**. Therefore, a protocol employing 10% DBU in DMF was applied for the deprotection of Fmoc-group by 2x10 seconds to suppress the DKP formation.[9] HR-TOF-MS was then used to ensure the presence of a molecular ion peak of hexadepsipeptide **13**. By using the later protocol, the Fmoc deprotection can avoid the DKP formation.

Further, the following steps are couplings of Fmoc-L-MeVal, Fmoc-L-Leu, and Fmoc-L-Pro, respectively, onto the peptidyl resin, which took advantage of a cocktail of HATU/HOAt/DIPEA in DMF as coupling reagent and DBU in DMF as deprotection agent until the linear nonadepsipeptide **16** was constructed. HR-TOF-MS analysis showed the presence of the correct molecular ion of the desired peptide at m/z 1089.6067 $[M+H]^+$.

After the linear nonadepsipeptidyl resin **16** was gained, the linear peptide was detached from the resin by using 20% TFA in DCM. From the solid-phase method, the crude linear of [Leu]^6-AbK **17** was obtained for 150 mg as pale yellow solid.

Cyclization of crude [Leu]^6-AbK **17** was undertaken by the employment of HATU/DIPEA as coupling reagent in dichloromethane over 48 h. The reaction was conditioned in a dilute concentation of 460

μM. The course of the reaction was monitored by TLC. Purification the crude cyclic [Leu]^6-AbK **1** was carried out by column chromatography using *n*-hexane:ethyl acetate as eluent, to gave 10 mg of cyclic product as white solid (5.31 %).



Scheme 2. Synthetic route of [Leu]^6-AbK 1. Reagents and conditions: (a) (1) Fmoc-L-MePhe, DIPEA, DCM, 18 h, rt; (2) 20% piperidine in DMF, 2+3+5 min. (b) (1) Fmoc-L-Phe, HATU/HOAt, DIPEA, DMF, 2×4 h, rt; (2) 20% piperidine in DMF, 2+3+5 min. (c) (1) Fmoc-L-MeVal, HATU/HOAt, DIPEA, DMF, 1×4 h, rt; (2) 20% piperidine in DMF, 2+3+5 min. (d) (1) Fmoc-D-Hiv, HATU/HOAt, DIPEA, DMF, 4×4 h, rt; (2) 20% piperidine in DMF, 2+3+5 min. (e) (1) Fmoc-L-MeVal, DIC/DMAP, DCM:DMF (95:5), 3×5 h, 45°C; (2) 20% piperidine in DMF, 2+3+5 min. (f) (1) Fmoc-L-Leu, HATU/HOAt, DIPEA, DMF, 2×4 h, rt; (2) 10% DBU in DMF, 2×10 s. (g) (1) Fmoc-L-MeVal, HATU/HOAt, DIPEA, DMF, 1×4 h, rt, (2) 10% DBU in DMF, 2×10 s. (h) (1) Fmoc-L-Leu, HATU/HOAt, DIPEA, DMF, 2×4 h, rt; (2) 10% DBU in DMF, 2×10 s. (i) (1) Fmoc-L-Pro, HATU, HOAt, DIPEA, DMF, 1×4 h, rt; (2) 10% DBU in DMF, 2×10 s. (j) 20% TFA in DCM, 2×10 min, rt. (k) HATU, DIPEA, DCM, 48 h, rt.

[Leu]^6-AbK **1** was analyzed by analytical RP-HPLC to confirm its purity (retention time = 6.49 min). Further, the cyclic peptide **1** was characterized by HR-TOF-MS and ^{1}H-NMR. A spectrum of HR-TOF-MS showed the correct molecular ion peak of the desired product with m/z 1093.6673 $[M+Na]^+$, corresponding to the calculated m/z 1093.6661. The ^{1}H-NMR spectra in CD_3OD revealed proton signals that were consistent for the structure of [Leu]^6-AbK **1**. The spectral data were compared to spectral data of AbA[15], AbE[16], and [2*S*,3*S*-Hmp]-AbL **2**[7], and the spectra clearly showed the presence of two conformers in a ratio of 58:42 for [Leu]^6-AbK **1** (**Table 1**). The presence of two conformers was also observed in the spectra of AbA **1** (57:43) and [2*S*,3*S*-Hmp]-AbL **2** (61:39) in $CDCl_3$. The conformer can occur since the influence of the solvent, and this phenomenon is commonly found in the spectroscopic NMR of peptide compounds[17].

Table 1. ^{1}H-NMR assignment in CD_3OD for conformer A and B of [Leu]^6-AbK **1**

Assignment	Conformer A	Conformer B
	^{1}H δ in ppm (ΣH, m, J in Hz)	
Hiv		
α-CH	4.98 (1H, t, 5 Hz)	5.00-5.03 (1H, m)
β-CH	2.07-2.09 (1H, m)	2.17-2.19 (1H, m)
γ-CH_3	0.74-0.82 (3H, m)	0.74-0.82 (3H, m)
γ-CH_3	0.83-0.92 (3H, m)	0.83-0.92 (3H, m)
MeVal1		
α-CH	5.34-5.39 (1H, m)	4.53-4.55 (1H, m)
β-CH	2.07-2.09 (1H, m)	2.17-2.19 (1H, m)
γ-CH_3	0.74-0.82 (3H, m)	0.74-0.82 (3H, m)
γ-CH_3	0.83-0.92 (3H, m)	0.83-0.92 (3H, m)
N-CH_3	2.57 (3H, s)	2.90 (3H, s)
Phe		
α-CH	5.76-5.80 (1H, m)	5.74-5.76 (1H, m)
β-CH	2.94-2.97 (1H, m)	2.92-2.94 (1H, m)
	2.76-2.80 (1H, m)	2.59-2.62 (1H, m)
C_δ (ar.)	7.04 (2H, d, 5 Hz)	7.11-7.28 (2H, m)
C_ε (ar.)	7.11-7.28 (2H, m)	7.11-7.28 (2H, m)
C_ζ (ar.)	7.11-7.28 (2H, m)	7.11-7.28 (2H, m)
NH	8.10 (1H, d, 8.2 Hz)	8.22 (1H, d, 5 Hz)
MePhe		
α-CH	2.76-2.92 (1H, m)	3.72-3.74 (1H, m)
β-CH	2.94-2.97 (1H, m)	2.94-2.97 (1H, m)
	2.76-2.80 (1H, m)	2.76-2.80 (1H, m)
C_δ (ar.)	7.04 (2H, d, 5 Hz)	7.04 (2H, d, 5 Hz)
C_ε (ar.)	7.11-7.28 (2H, m)	7.11-7.28 (2H, m)
C_ζ (ar.)	7.11-7.28 (2H, m)	7.11-7.28 (2H, m)
N-CH_3	2.72 (3H, s)	2.16 (3H, s)
Pro		
α-CH	4.17 (1H, t, 8.1 Hz)	4.10 (1H, d, 5 Hz)
β-CH	3.00-3.05 (2H, m)	2.48-2.52 (2H, m)
γ-CH_2	1.26-1.36 (1H, m)	1.26-1.36 (1H, m)
	1.13-1.17 (1H, m)	1.13-1.17 (1H, m)
δ-CH_2	3.20-3.24 (1H, m)	3.20-3.24 (1H, m)
	3.23-3.30 (1H, m)	3.23-3.30 (1H, m)
Leu		
α-CH	4.49-4.55 (1H, m)	4.49-4.55 (1H, m)
β-CH_2	0.96-1.14 (1H, m)	0.96-1.14 (1H, m)
	1.26-1.36 (1H, m)	1.26-1.36 (1H, m)
γ-CH	1.26-1.36 (1H, m)	1.26-1.36 (1H, m)
δ-CH_3	0.74-0.82 (3H, m)	0.74-0.82 (3H, m)
δ-CH_3	0.83-0.92 (3H, m)	0.83-0.92 (3H, m)
NH	7.90 (1H, d, 7.8 Hz)	8.04 (1H, d, 8.0)
MeVal2		
α-CH	5.66 (1H, t, 11.4)	5.66 (1H, t, 11.4)
β-CH	2.07-2.09 (1H, m)	2.17-2.19 (1H, m)
γ-CH_3	0.74-0.82 (3H, m)	0.74-0.82 (3H, m)
γ-CH_3	0.83-0.92 (3H, m)	0.83-0.92 (3H, m)
N-CH_3	3.06 (3H, s)	3.01 (3H, s)
Leu		
α-CH	4.50-4.53 (1H, m)	4.50-4.53 (1H, m)
β-CH_2	0.96-1.14 (1H, m)	0.96-1.14 (1H, m)
	1.26-1.36 (1H, m)	1.26-1.36 (1H, m)
γ-CH	1.26-1.36 (1H, m)	1.26-1.36 (1H, m)
δ-CH_3	0.74-0.82 (3H, m)	0.74-0.82 (3H, m)
δ-CH_3	0.83-0.92 (3H, m)	0.83-0.92 (3H, m)
NH	7.65 (1H, d, 7.8 Hz)	7.77 (1H, d, 8.1 Hz)
MeVal3		
α-CH	5.20 (1H, t, 11.2 Hz)	5.20 (1H, t, 11.2 Hz)
β-CH	2.07-2.09 (1H, m)	2.17-2.19 (1H, m)
γ-CH_3	0.74-0.82 (3H, m)	0.74-0.82 (3H, m)
γ-CH_3	0.83-0.92 (3H, m)	0.83-0.92 (3H, m)
N-CH_3	2.67 (3H, s)	2.62 3H, s)

3. Conclusions

[Leu]^6-AbK **1** has been successfully synthesized using a combination of solid- and solution-phase methods. This new method has increased the overall yield from 1.89% ([2*S*,3*S*-Hmp] AbL **2**) to 5.31%. An ester bond was constructed on the resin using DIC/DMAP as the coupling agent that was applied at 45°C through Steglich esterification. An incomplete reaction between Hiv-OH and Fmoc-MeVal is

found to be the main issue of the synthesis that has resulted in the low yield of the product. The presence of β-branch structures from both sides of the residues has made the ester bond formation difficult.

Acknowledgements

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4. Experimental

4.1. Materials and Methods

The chemical used in this research were 2-chlorotrityl chloride resin, dichloromethane, dimethylformamide (DMF), *N,N*-diisopropyletilamine (DIPEA), *N,N'*-diisopropylcarbodiimide (DIC), D-valine, Fmoc-chloride, Fmoc-L-MePhe-OH, Fmoc-Phe-OH, Fmoc-L-MeVal-OH, Fmoc-L-Leu-OH, Fmoc-L-Pro-OH, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium3-oxide hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), 4-dimethylaminopyridine (DMAP), trifluoroacetic acid (TFA), methanol, tetrahydrofuran (THF), toluene, *n*-hexane, ethy acetate, hydrochloric acid, sulphuric acid, sodium nitrite, sodium sulphate, pyridine, piperidine and 1,8-diazobicyclo(5.4.0)undecan-7-ene (DBU). All materials used are proanalyst. Amino acids and resin were purchased from GL-Biochem, Shanghai, China. The instrumentations used for synthesis were SPPS tubes, rotary evaporator, shaking rotator, analytical reverse-phase high performance liquid chromatography (RP-HPLC) (Waters 2998 Photodiode Array Detector, LiChrospher 100, column C-18 5 mm), mass spectrometer (Waters HR-TOF-MS Lockspray), UV-Vis spectrophotometer (TECAN Infinite pro 200), automatic polarimeter (ATAGO AP300) and nuclear magnetic resonance (NMR) spectroscopy (Agilent 500 MHz).

4.2. General procedure

4.2.1 **Synthesis of *(R)*-2-hydroxy-3-methylbutanoic acid 5**[12]

D-valine **4** (4 g, 34.18 mmol) was dissolved in 1 N sulfuric acid (100 mL). This solution was coolled into 0°C , and was added by a solution of sodium nitrite in distilled water (40 mL) dropwisely (14.15 g, 205.08 mmol) over 2 h (the temperature was maintained). The mixture was allowed to warm up to room temperature and stirred at this temperature for 17 hrs (a completion of reaction was guided by thin layer chromatography). The reaction mixture was saturated by sodium chloride and was then extracted by ethyl acetate (3×25 mL). The organic fraction was dried by sodium sulphate, filtered and concentrated.

4.2.2 **Synthesis of *(R)*-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)oxy)-3-methylbutanoic acid 7**[8]

To the solution of D-Hiv **5** (736 mg, 6.24 mmol) and Fmoc-Cl **6** (1,690 mg, 6.55 mmol) in THF was added pyridine (518 µL, 6.55 mmol) dropwisely in THF (2 mL) at 0°C over 20 min (the temperature was maintained). The mixture was stirred over 2 h at 0-5°C, and then allowed to warm up to the room temperature and stirred over 17 h. The reaction was concentrated, the crude product was dissolved in dichloromethane and washed with 1 N HCl and water, successively. The organic phase was dried by sodium sulphate and filtered. The filtrate was concentrated to give the crude as a yellow solid, the crude was cyrstallized from toluene.

4.2.3 **Solid-phase method**

Loading the resin. 2-chlorotritylchloride resin (300 mg, capacity: 0.98 mmol/g) was swollen in dichloromethane for 15 min. Attachment of Fmoc-L-MePhe onto resin was performed by the addition of a solution of Fmoc-L-MePhe (180 mg, 0.45 mmol) and DIPEA (157 µL, 0.90 mmol) dissolved in dichloromethane (3 mL, overnight). The resin was then filtered and washed with dichloromethane (loading resin: 0.60 mmol/g). To the resin was added a mixture of dichloromethane:methanol:DIPEA

(80:15:5, 3 mL) and the mixture was swollen for 2x10 min. The resin was then filtered and washed with DMF dan dichloromethane, successively.

Fmoc deprotection 1. Fmoc-peptidyl resin was shaken with piperidine 20% v/v in DMF (2 mL) for 2+3+5 min. The resin was then filtered and washed with DMF and dichloromethane, respectively. To a small portion of dry resin beads was added a chloranil test solution for the confirmation of the presence of the exposed primary amine or *N*-methylated amino acid.

Fmoc deprotection 2.[9] Fmoc-peptidyl resin was shaken with DBU 10% v/v in DMF (3 mL) for 2x10 sec. The resin was then filtered and washed with DMF and dichloromethane, successively. To a small portion of dry resin beads was added a chloranil test solution for the confirmation of the presence of the exposed primary amine or *N*-methylated amino acid.

HATU/HOAt-mediated coupling.[14] To the dry depsipeptidyl resin having been swollen by DMF (3 mL) was added Fmoc-amino acid (0.54 mmol) that was previously treated with HATU (205 mg, 0.54 mmol), HOAt (73 mg, 0.54 mmol) and DIPEA (188 µL, 1.08 mmol). The resin with primary amine end was shaken for 4 h and the resin with *N*-methylated amino acid end was shaken for 2x4 h. The resin was then filltered and washed with DMF and dichloromethane, successively. To a small portion of dry resin beads was added a chloranil test solution for the confirmation of the presence of the exposed primary amine or *N*-methylated amino acid.

DIC/DMAP-mediated coupling.[11] The resin was transferred to a culture tube provided with a magnetic stirrer. To the solution of Fmoc-L-MeVal (318mg, 0.90 mmol) in THF (2,5 mL) was added DIC (144 µL, 0.62 mmol) and 10% mol of DMAP in DMF (0.5 mL). The mixture was reacted for 3x5 h at 45°C with smooth stirring (the temperature was maintained). The reaction mixture was transferred to the syringe, the solvents removed by filtration and the resin washed with DMF and dichloromethane, successively. The success of the ester bond formation was monitored by taking a small portion of dry resin that was then added by 2%TFA v/v in DCM (100 µL), and analysed by HR-TOF-MS.

Resin cleavage. To the depsinonapeptidyl-resin was added a cleavage cocktail of 20% TFA in dichloromethane (5 mL). The resin was then shaken for 2x10 min. The resin was washed with dichloromethane. The combined solution was evaporated and the resulting crude was obtained as a pale yellow solid.

4.2.4 Cyclization procedure trough solution-phase

Crude of linear depsipeptide (150 mg) was dissolved in dichlorometane (150 mL). To the solution were added HATU (0.55 mmol) in DMF (100 µL) and DIPEA (1.38 mmol). The mixture was stirred over 48 h in room temperature (followed by TLC to completion). The reaction mixture was concentrated to give crude as a dark-brown oil. The crude was dissolved in ethyl acetate (100 mL) and then was extracted with brine solution (3×30 mL). The ethyl acetate was combined and evaporated to give the resulting crude as a yellow solid. The resulting crude was purified by silica gel column chromatography (*n*-hexane/ethyl acetate=1:1) to obtain the cyclic product.

4.3 Physical and Spectral Data

4.3.1 **Synthesis of *(R)*-2-hydroxy-3-methylbutanoic acid 5**: Pale yellow solid (3.01 g, 88.2 %): $[\alpha]_D^{28}$ = +86.0, in dichloromethane; IR (KBr) ν_{max} (cm^{-1}) 3425 (OH hydroxyl), 2917 (aliphatic CH), 1722 (C=O carboxylic group), 1391, 1373 (gem dimethyl), 1135 (C-O); ^{1}H NMR (500 MHz, CD_3OD) δ_H (ppm) 5.13 (br, 2H, O*H* and COO*H*), 3.95 (d, *J* = 4.2 Hz, 1H, OHC*H*COOH), 2.12 – 1.98 (m, 1H, CH_3CHCH_3), 1.01 (d, *J* = 6.9 Hz, 3H, CH_3CHCH_3), 0.92 (d, *J* = 6.9 Hz, 3H, CH_3CHCH_3)

4.3.2 **Synthesis of *(R)*-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)oxy)-3-methylbutanoic acid 7**: White solid (773 mg, 51%): $[\alpha]_D^{28}$ = +70.0, in dichloromethane; IR (KBr) ν_{max} (cm^{-1}) 3400-2800 (OH carboxylic group), 3066 (aromatic CH), 2970 (aliphatic CH), 1752 (C=O carboxylic group), 1716 (O(C=O)O), 1665 (aromatic CH), 1260, 1229, 1280 (C-O), 738 (disubstituted aromatic); ^{1}H NMR (500

MHz, $CDCl_3$) δ_H (ppm) 7.77 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.64 (dd, *J* = 13.8, 7.4 Hz, 2H, Ar*H*), 7.41 (t, *J* = 7.5 Hz, 2H, Ar*H*), 7.32 (t, *J* = 7.4 Hz, 2H, Ar*H*), 4.88 (d, *J* = 4.2 Hz, 1H, ArC*H*Ar), 4.52 (dd, *J* = 10.2, 7.1 Hz, 1H, O(C=O)OC*H*COOH), 4.34 (dt, *J* = 14.8, 7.7 Hz, 2H, CHCH_2O(C=O)O), 2.44 – 2.26 (m, 1H, CH_3CHCH_3), 1.11 (d, *J* = 6.9 Hz, 3H, CH_3CHCH_3), 1.09 (d, *J* = 6.9 Hz, 3H, CH_3CHCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) 174.37, 155.06, 143.56, 143.24, 141.45, 141.44, 128.08, 128.05, 127.38, 127.33, 125.45, 125.32, 120.20, 79.54, 77.16, 70.58, 46.83, 30.35, 18.84, 17.18.

4.3.3 **[Leu]^6-AbK 1**: White solid (10 mg, 5.31 %). Mass spectrum: *m/z* 1093.6673 $[M+Na]^+$. $C_{59}H_{90}N_8O_{11}$. Calculated 1093.6661. ^{1}H-NMR in CD_3OD (500 MHz): See **Table 1**.

References

1 Davies J. S. (**2003**) The cyclization of peptides and depsipeptides. *J. Pept. Sci.,* 9 (3) 471–501.
2 Awazu N., Ikai N., Yamamoto J., Nishimura K., Mizutani S., Takesako K., and Kato I. (**1994**) Structures and antifungal activities of new aureobasidins. *J. Antibiot.,* 48 (6) 525–527.
3 Takesako K., Mizutani S., Sakakibara H., and Endo M. (**1996**) Precursor directed biosynthesis of aureobasidins. *J. Antibiot.,* 49 (7) 676–681.
4 Kurome T., Inami K., Inoue T., Ikai K., Takesako K., Kato I., and Shiba T. (**1996**) Total synthesis of an antifungal cyclic depsipeptide aureobasidin A. *Tetrahedron.,* 52 (12) 4327–4346.
5 Jao E., Cooper A. B., Rane D. F., Saksena A. K., Desai J., and Road, G. H. (**1996**) Total synthesis of the antifungal cyclic depsipeptides Sch 57697 and aureobasidin A. *Tetrahedron Lett.,* 37 (32) 5661–5664.
6 Schmidt U., Schuacher A., Mittendorf J., and Riedl B. (**1998**) Contribution to the synthesis of aureobasidin A . Synthesis of cyclopep tolides containing the sequence 1eucyl-*N*-Ph. *J. Pept. Res.,* 52 (2) 143–154.
7 Maharani R., Brownlee R. T. C., Hughes A., and Abbott B. A. (**2014**) Total synthesis of a highly *N*-methylated cyclodepsipeptide [2*S*,3*S*-Hmp]-aureobasidin L using solid-phase methods. *Tetrahedron.,* 70 (14) 2351–2358.
8 Kuisle O., Quinoa E., and Riguera R. A. (**1999**) General methodology for automated solid-phase synthesis of depsides and depsipeptides. preparation of a valinomycin analogue. *J. Org. Chem.,* 64 (9) 8063–8075.
9 Coin I., Beerbaum M., Schmieder P., Bienert M., and Beyermann M. (**2008**) Solid-phase synthesis of a cyclodepsipeptide : cotransin. *Org. Lett.,* 10 (17) 3857–3860.
10 Nguyen M. M., Ong N., and Suggs L. A. (**2013**) General solid phase method for the synthesis of depsipeptides. *Org. Biomol. Chem.,* 11 (7) 1167–1170.
11 Gimeno M. P., Ramos Y. G., Martin M. J., Spengler J., Guijarro J. M., Munt S., Francesch A. M., Cuevas C., Puche J. T., and Albericio F. (**2013**) The first total synthesis of the cyclodepsipeptide. *Nat. Commun.,* 4 (2352) 1–10.
12 Müller J., Feifel S. C., Schmiederer T., and Zocher R. (**2009**) In vitro synthesis of new cyclodepsipeptides of the pf1022-type : probing the α-d-hydroxy acid tolerance of pf1022 synthetase. *ChemBioChem.,* 10 (2) 323–328.
13 Zhang M., Rodriguez R. M., Lacey E., Piggott A. M., Leung I. K. H., and Brimble M. A. (**2017**) Cyclization of linear tetrapeptides containing *N*-methylated amino acids by using 1-propanephosphonic acid anhydride. *Eur. J. Org. Chem.,* 2017 (1) 149–158.
14 Angell M., Garcia-Echeverria C., and Rich H. (**1994**) Comparative studies of the coupling of *N*-methylated, sterically hindered amino acids during solid-phase peptide synthesis. *Tetrahedron Lett.,* 35 (33) 5981–5984.
15 Ikai K., Takesako K., Shiomi K., Moriguchi M., Umeda Y., Yamamoto J., and Kato I., (**1991**) Structure of aureobasidin A. *J. Antibiot.,* 44 (11) 925–933.
16 Fujikawa A., In Y., Inoue M., and Ishida T. (**1994**) X-ray and NMR conformational study of aureobasidin E: a cyclic depsipeptide with potent antifungal activity. *J. Org. Chem.,* 59 (3) 570–578.
17 Kessler H. (**1982**) Conformation and biological activity of cyclic peptides. *Angew. Chem. Int. Ed. Engl.,* 21 (7) 512–523.