Synthesis of *t*-Butyl (2*R*)-Hydroxyisovalerate, A Precursor of Aureobasidin B

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Abstract. Aureobasidins are a family of cyclodepsipeptides have antifungal properties. They were isolated from the black yeast *Aureobasidium pullulans* R106 and over 30 derivatives have been successfully characterized. There are few publications reporting the total synthesis of aureobasidins. The limited reports of the synthesis of the aureobasidin derivatives are due to the difficult access to the preparations of precursors. The aim of this research is to synthesise a precursor of aureobasidin B, *t*-butyl (2*R*)-hydroxyisovalerate (*t*-Bu-Hiv), that is prepared for the total synthesis of aureobasidin B. The synthesis of AbB is planned to be undertaken by using a solid phase method, so the ester formation between *t*-Bu-Hiv and the Fmoc-β-hydroxymethylvaline will be carried out in solution phase to form depsidipeptide. The *t*-butyl group was used as protecting agent that is due to the straightforward elimination of the protecting group from the Fmoc-depsidipeptide. The *t*-Bu-Hiv acid was prepared from D-valine through diazotisation to form (2*R*)-acetyloxyisovaleric acid in 62.7% yield. Product of the first step was then protected by *t*-butyl group by using Boc-anhydride in *t*-butanol to give *t*-butil (2*R*)-acetyloxyisovalerate in 44% yield. In the last step, the acetyloxy group was eliminated by using potassium carbonate in methanol/water to give the desired product, *t*-Bu-Hiv in 33.5% yield. The *t*-Bu-Hiv is ready to be combined with Fmoc-β-hydroxymethylvaline to result in depsidipeptide that will be attached to the resin in the total synthesis of AbB. Each stage of this synthesis was controlled by thin layer chromatography and all products were purified by open column chromatography. All the synthesized products were characterized by various spectroscopic techniques, including infrared spectrophotometer, mass spectroscopy (ESI-MS), ^{1}H-NMR and ^{13}C-NMR.

1. Introduction

Aureobasidins, isolated from the black yeast *Aureobasidium pullulans* R106, are highly *N*-methylated cyclodepsipeptides, which have antifungal activity. Over 30 aureobasidin analogues have been successfully characterized including the major compound, aureobasidin A (AbA) (Figure 1). All of the aureobasidins are cyclic depsipeptides consisting of eight amino acids in which three or four of them are *N*-methylated and one alpha hydroxy acid is always present. All of the amino acids have the L-configuration.(1-3)

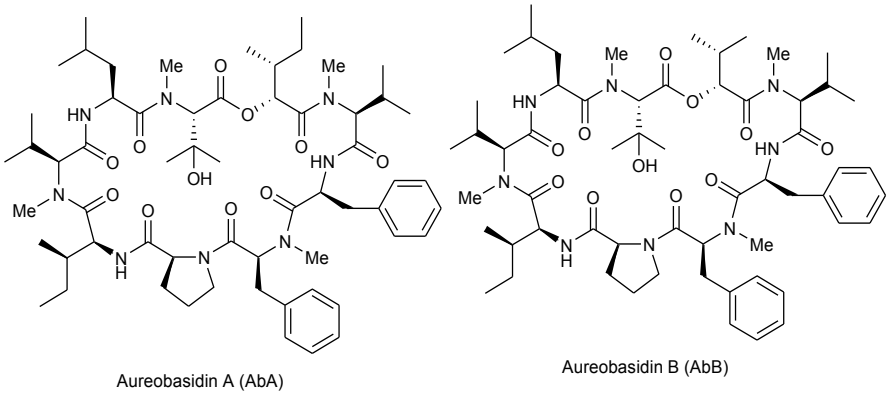

Figure 1. Structures of aureobasidin A (AbA) and aureobasidin B (AbB) .

There were few papers reporting total synthesis of aureobasidin compounds. In the 1990s, three articles described total synthesis of AbA in solution phase and another article was reported in 2014 for total synthesis of analogue of AbL, [2S,3S-Hmp]-AbL, carried out in a combination of solution- and solid-phase synthesis.(4-7) These few reported synthesis showed that the access into the aureobasidins was limited. One of the issues following the synthesis are the presence of some unusual residues that could be either commercially available with expensive prices or commercially unavailable and would need to be synthesized. Some of these residues are N-methylated amino acids and hydroxy acids. The later one would be our focused topic, particularly in preparation of the hydroxy acid.

Hydroxy acids, such as 2-hydroxy-2-methylpentanoic acid (D-Hmp) and 2-hydroxyisovaleric acid (Hiv), are moieties often found in aureobasidins and access to these types of compounds has been reported.(8-11) In this present work, Hiv as a residue of AbB (Figure 1) was being a target of synthesis, providing residue for the total synthesis of AbB that has not been synthesized priorly. A *t*-Bu-protected Hiv, was explored as this protecting group can be easily removed in acidic conditions. This strategy had been employed by Sleebs *et al*. (2011), when *t*-butyl L-phenyllactate was prepared for coupling to Fmoc-L-pipecolic acid under Mitsunobu conditions and by Maharani *et al*. (2014), when *t*-butyl (2*S*)-hydroxy-(3*S*)-methylpentanoate was prepared for coupling to Fmoc-MeVal under Steglich esterification.(7, 12)

2. Methodology

Methodology for the synthesis of *t*-butyl (2*R*)-hydroxyisovalerate was carried out through a methodology applied for the synthesis of *t*-butyl (2*S*)-hydroxy-(3*S*)-methylpentanoate.(7)

2.1. Formation of (2R)-acetyloxyisovaleric acid

D-valine (2 g;17.1 mmol) was dissolved in glacial acetic acid (25.6 mL). The stirred solution was occasionally cooled to keep the solution at room temperature. To this solution was added sodium nitrite (2.35 g;34 mmol) in several portions over 1 h. Once the addition was complete, the solution was stirred for 24 h. The reaction mixture was then evaporated and the crude was dissolved in ether. The ether solution was washed with water several times and then extracted with saturated sodium bicarbonate solution. The combined aqueous solution was acidified with 2 N hydrochloric acid and then extracted with ether. The organic layer was dried and evaporated to give (2*R*)-acetyloxy isovaleric acid (62.7%) as a colorless oil.

2.2. Formation of t-butyl (2R)-acetyloxyisovalerate

To a solution of (2*R*)-acetyloxy isovaleric acid (1.5 g;9.38 mmol) in *t*-butanol (5.95 mL) was added di-*tert*-butyldicarbonate (2.57 g;11.17 mmol) and 4-dimethylaminopyridine (0.14g;1.17 mmol). The

solution was stirred under nitrogen at room temperature for 4 h with the progress of reaction was monitored by TLC. The reaction mixture was evaporated and the resulting crude was dissolved in ethyl acetate. The ethyl acetate solution was washed with saturated sodium bicarbonate solution, dried with $MgSO_4$ and evaporated. The crude was purified by silica gel column chromatography (hexane:ethyl acetate = gradient eluent) to give pure *t*-butyl (2*R*)-acetyloxyisovalerate (43.9%) as a colorless oil.

2.3. Formation of t-butyl (2R)-hydroxyisovalerate

Compound *t*-butyl (2*R*)-acetyloxyisovalerate (0.2 g;67.5 mmol) was dissolved in water (3.39 mL) and methanol (2.54 mL). To this solution was added potassium carbonate (0.5 g;3.64 mmol) and the solution was stirred for 18 h with monitoring by TLC. The reaction mixture was evaporated and the resulting crude was evaporated and partitioned between water and ether. The organic layer was dried and evaporated to give *t*-butyl (2*R*)-hydroxyisovalerate (33.5%) as a colorless oil.

2.4. Characterizations of synthesized products

All of the synthesized products either intermediates or final products were characterized by spectroscopic methods, ESI-MS, IR, ^{1}H- and ^{13}C-NMR.

3. Results and Discussion

Preparation of *t*-butyl (2*R*)-hydroxyisovalerate started from a conversion of D-Val into its α-acetyloxy acid. According to a protocol explained by Kolasa and Miller, the deaminative hydroxylation of D-Val resulted in acetate.(13) The diazotisation reaction was carried out in the presence of glacial acetic acid/$NaNO_2$ generating 62.7% yield of the desired product (Figure 2). The product was characterized by ESI-MS, IR and NMR. ESI-MS analysis showed correct molecular mass of the acetate with $[M+H]^+$ 161,0609, representing the desired product. This data was supported by IR analysis showing the presence of broad O-H stretch with υ_{max} 3310 cm^{-1} that is characteristic for –COOH group. Two C=O stretches at υ_{max} 1750 and 1730 cm^{-1} showed the existence of two carbonyl groups of ester and carboxylic groups, respectively. The presence of a signal at δ_H 2.15 ppm in the ^{1}H-NMR represented methyl protons in the acetate moiety. In addition, the presence of one additional carbonyl signal (δ_C 171.4 ppm) showed that the acetate was present in the structure.

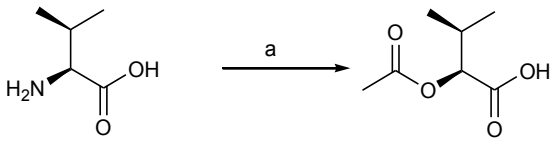

Figure 2. Synthesis of (2*R*)-acetyloxyisovaleric acid, (a) acetic acid, $NaNO_2$.

A mechanistic study by Sleebs describes the active intermediate, a diazonium ion, as being attacked by acetic acid instead of water (Figure 3).

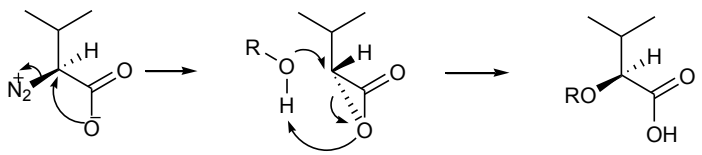

Figure 3. Mechanism for α-hydroxy carboxylic acid formation (R = H or R = Ac).

The *t*-Bu protective group was introduced to the (2*R*)-acetyloxyisovaleric acid by the addition of *t*-butanol, $(Boc)_2O$ and a catalyst, DMAP, in 43.9% yield (Figure 4). The structural characterisation of the resulting compound was based on ESI-MS, IR and NMR. ESI-MS analysis provided the molecular mass ion of *m/z* 217.0182, indicating the $[M+H]^+$ ion of the desired product, *t*-butyl (2*R*)-acetyloxyisovalerate. The absence of wavenumber of –COOH group has also supported that the carboxylic group has been successfully esterified. Nine upfield protons at δ_H 1.46 ppm in the ^{1}H-NMR with a singlet multiplicity were assigned as the three methyls corresponding to the *t*-butyl protective group. A downfield quaternary carbon at δ_C 82.0 ppm also indicated the presence of this group.

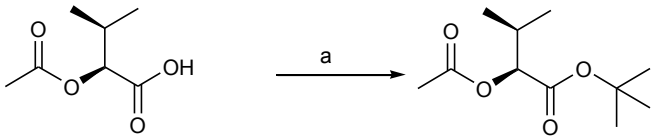

Figure 4. Synthesis of *t*-butyl (2*R*)-acetyloxyisovalerate, (a) *t*-butanol, $(Boc)_2O$, DMAP.

Once the *t*-butyl group was attached to the Hiv-acetate, the next task was to remove the acetyl group affording the corresponding *t*-butyl (2*R*)-hydroxyisovalerate. Through a simple deprotection reaction under basic conditions (K_2CO_3 in 50% MeOH in water), *t*-butyl (2*R*)-hydroxyisovalerate was afforded in 33.5% yield (Figure 5). The ESI-MS spectrum showed molecular mass ion of *m/z* 175,1189, indicating $[M+H]^+$ of the final product, *t*-butyl (2*R*)-hydroxyisovalerate (Figure 6). This data was supported by the IR spectrum, showing the presence of O-H stretch with wave number of 3517 cm^{-1} (Figure 7). This indicated that the acetyl group had been removed leaving the hydroxyl group. The successful reaction was also characterized by the loss of acetate signals in the ^{1}H-NMR and ^{13}C-NMR spectra (Figure 8 and 9).

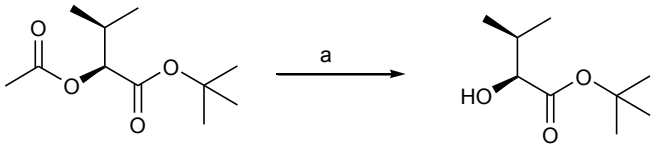

Figure 5. Acetate removal, (a) K_2CO_3 in 50% MeOH in water.

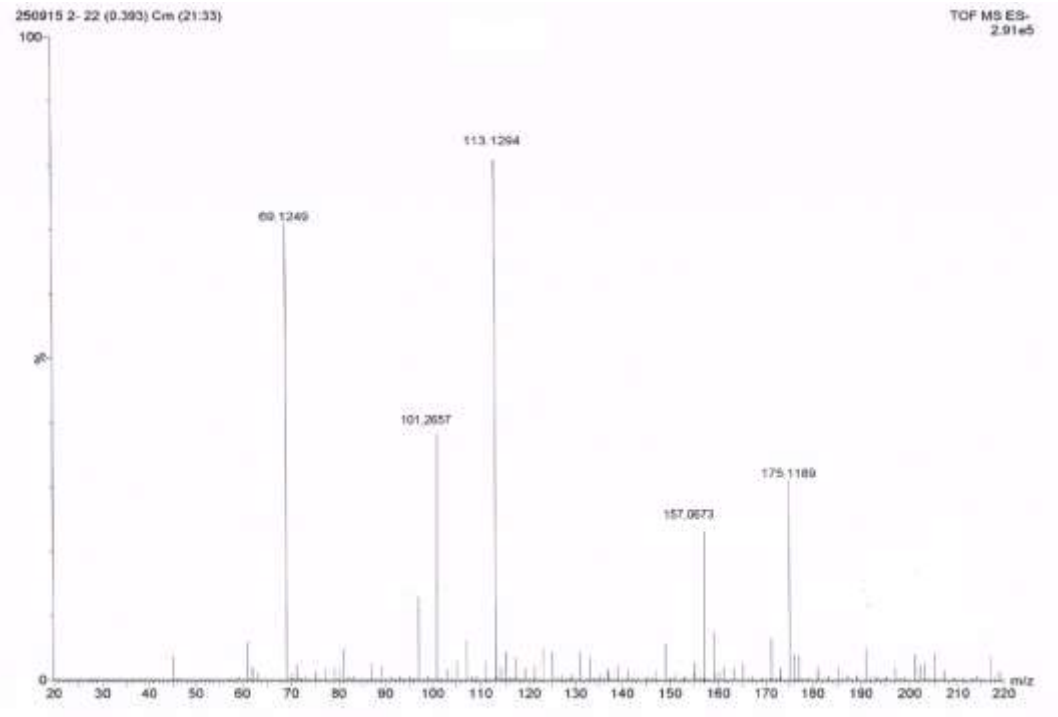

Figure 6. Mass spectrum of *t*-butyl (2*R*)-hydroxyisovalerate.

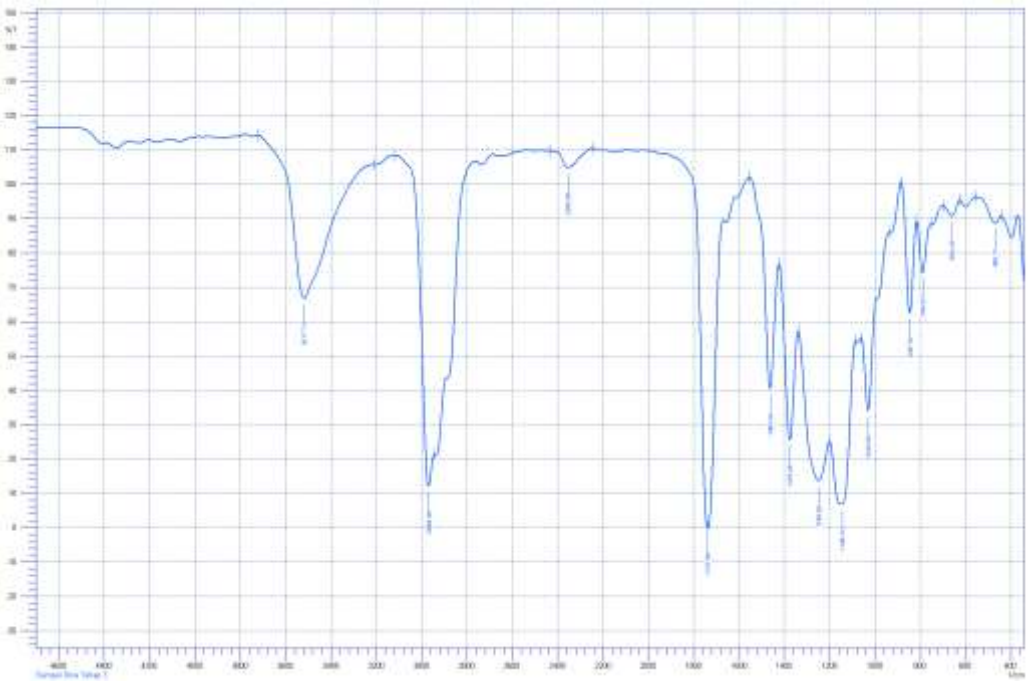

Figure 7. The infrared spectrum of *t*-butyl (2*R*)-hydroxyisovalerate.

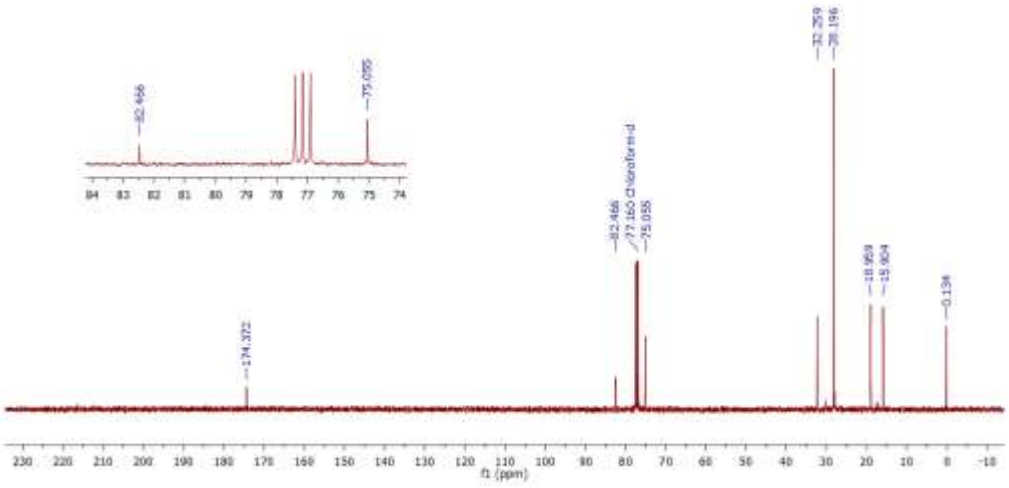

Figure 8. ^{13}C-NMR spectrum of *t*-butyl (2*R*)-hydroxyisovalerate.

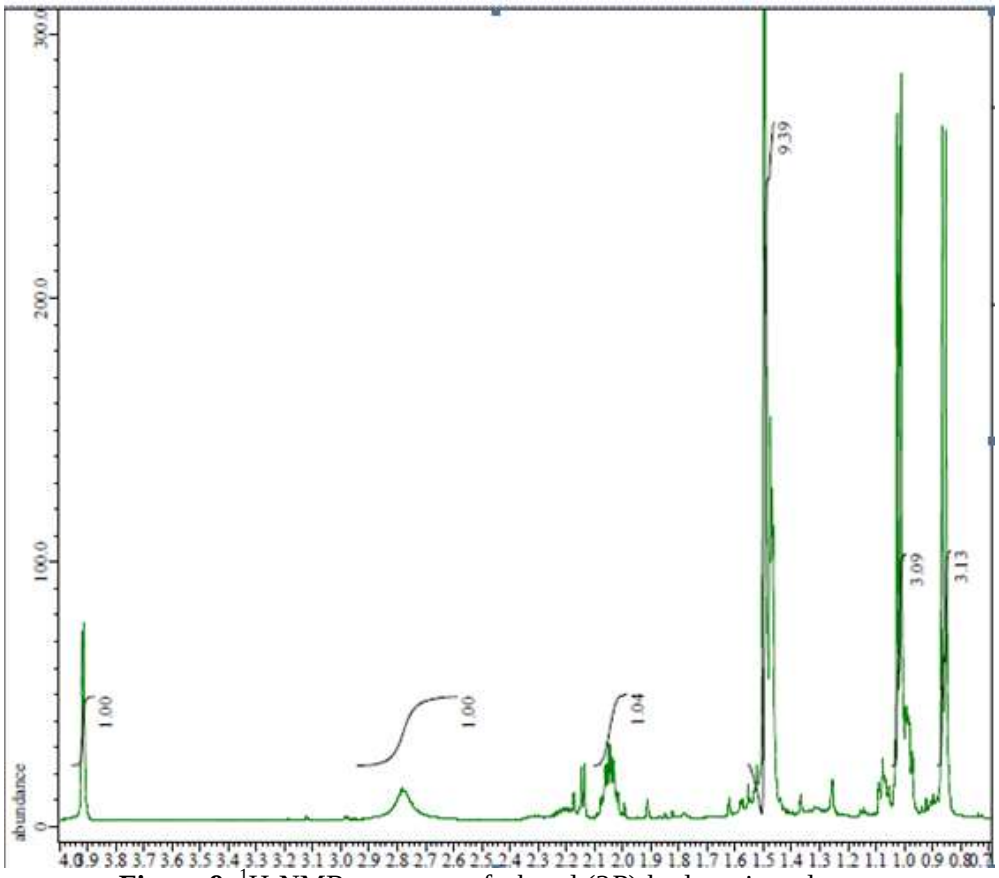

Figure 9. ^{1}H-NMR spectrum of *t*-butyl (2*R*)-hydroxyisovalerate.

4. Conclusion

Compound *t*-butyl (2*R*)-hydroxyisovalerate has been successfully synthesized through three consecutive steps, diazotisation, t-Bu attachment and acetate deprotection with 62.7%, 43.9%, and 33.5% yields, respectively.

All of the synthesized products, either intermediates or final product, were characterized by spectroscopic methods, IR, ESI-MS and NMR.

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References

1. Turner W and Rodriguez M 1996 *Current Pharmaceutical Design* **2(2)** 209-24.
2. Awazu N, Ikai K, Yamamoto J, Nishimura K, Mizutani S, and Takesako K 1995 *The Journal of antibiotics* **48(6)** 525-7.
3. Takesako K, Mizutani S, Sakakibara H, Endo M, Yoshikawa Y, and Masuda T 1996 *The Journal of antibiotics* **49(7)** 676-81.
4. Kurome T, Inami K, Inoue T, Ikai K, Takesako K, and Kato I 1996 *Tetrahedron* **52(12)** 4327-46.
5. Jao E, Cooper AB, Rane DF, Saksena AK, Desai J, and Wang J 1996 *Tetrahedron letters* **37(32)** 5661-4.
6. Schmidt U, Schumacher A, Mittendorf J, and Riedl B 1998 *The Journal of peptide research* **52(2)** 143-54.
7. Maharani R, Brownlee RT, Hughes AB, Abbott BM 2014 *Tetrahedron* **70(14)** 2351-8.
8. Snowden RL, Grenno R, and Vial C 2005 *Flavour and fragrance journal* **20(4)** 372-80.
9. Elgersma RC, Mulder GE, Posthuma G, Rijkers DT, and Liskamp RM 2008 *Tetrahedron Letters* **49(6)** 987-91.
10. Zubia A, Mendoza L, Vivanco S, Aldaba E, Carrascal T, and Lecea B 2005 *Angewandte Chemie* **117(19)** 2963-7.
11. Koh JT, Cornish VW, and Schultz PG 1997 *Biochemistry* **36(38)** 11314-22.
12. Sleebs MM, Scanlon D, Karas J, Maharani R, and Hughes AB 2011 *The Journal of organic chemistry* **76(16)** 6686-93.
13. Kolasa T and Miller MJ 1987 *The Journal of Organic Chemistry* **52(22)** 4978-84.