Chimica et Natura Acta
p-ISSN: 2355-0864 e-ISSN: 2541-2574
Homepage: http://jurnal.unpad.ac.id/jcena

Synthesis of α-Hydroxyisovaleric acid (Hiv) and α-Acetyloxyisovaleric Acid (Ac-Hiv), Precursors of Aureobasidin B, with Improved Yield

Rani Maharani[1,2,*], Andi Rahim[1], Herdanu Rizqullah[1], Nur Muhammad Miftah[1], Ace Tatang Hidayat[1,2], Achmad Zainuddin[1], Nurlelasari[1], Desi Harneti[1], Unang Supratman[1,2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang km. 21 Jatinangor, West Java, Indonesia 45363
[2]Laboratorium Sentral, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang km. 21 Jatinangor, West Java, Indonesia 45363
*Corresponding author: r.maharani@unpad.ac.id

DOI: https://doi.org/10.24198/cna.v6.n3.20855

Abstract: α-Hydroxyisovaleric acid (Ac-Hiv) and α-acetyloxyisovaleric acid (Ac-Hiv) have been successfully synthesized through a diazotisation of amino acid using sodium nitrite with the catalyst of sulfuric acid and acetic acid, respectively. In the synthesis of Hiv, Zubia *et al*. (2005) mentioned that 3 equivalents of sodium nitrite for the reaction gave the hydroxy acid with a good yield. However, Cohen-Arazi *et al.* (2008) described that 6 equivalents of sodium nitrite resulted the highest yield. In present study, a variation of equivalents of sodium nitrite (3, 4, 5, 6 eq.) were trialed for the same method of synthesis. Through several experiments, we found that 6 equivalents of sodium nitrite were the best portion among all. This finding was applied into the synthesis of protected Hiv (Ac-Hiv) that was previously reported by Maharani *et al.* (2017) giving 63% yield when 3 equivalent of sodium nitrite was employed. By increasing the equivalent of sodium nitrite into 6 equivalents, the Ac-Hiv can be synthesized with an improved yield (71%).

Keywords: α-acetyloxyisovaleric acid (Ac-Hiv), diazotization, α-hydroxyisovaleric acid (Ac-Hiv), hydroxy acid

***Abstract*:** *Asam α-hidroksiisovalerat (Ac-Hiv) dan asam α-asetiloksiisovalerat (Ac-Hiv) telah disintesis menggunakan diazotisasi asam amino dengan menggunakan natrium nitrit dengan katalis asam sulfat dan asam asetat, berturut-turut. Dalam sintesis Hiv, Zubia et al. (2005) menyebutkan bahwa penggunaan 3 ekuivalen natrium nitrit dalam reaksi akan memberikan perolehan hasil yang baik. Akan tetapi, Cohen-Arazi et al. (2008) menjelaskan bahwa 6 ekuivalen natrium nitrit akan memberikan hasil terbaik. Dalam studi saat ini, variasi ekuivalen dari natrium nitrit (3, 4, 5, 6 eq.) akan dilakukan dengan menggunakan metode sintesis yang sama yang dilaporkan oleh Zubia et al. (2005) dan Cohen-Arazi et al. (2008). Dari percobaan yang dilakukan, kami menemukan bahwa 6 ekuivalen natrium nitrit merupakan komposisi terbaik untuk reaksi ini. Penemuan ini diterapkan dalam sintesis Hiv yang terproteksi asetil (Ac-Hiv) yang sebelumnya telah dilaporkan oleh Maharani et al. (2017) dengan perolehan hasil sebesar 63% ketika 3 ekuivalen natrium nitrit diaplikasikan. Dengan meningkatkan ekuivalen dari natrium nitrit sebanyak dua kali lipat, telah meningkatkan perolehan hasil Ac-Hiv menjadi 71%.*

Kata kunci: *Asam α-asetiloksiisovalerat (Ac-Hiv), asam hidroksi, asam α-hidroksiisovalerat (Ac-Hiv), diazotisasi*

INTRODUCTION

α-Hydroxy acids are a class of compounds that are always present in depsipeptides (Luesch *et al*. 2002; Sasaki *et al*. 1992; Suzuki *et al*. 1970; Tomoda *et al*. 1992). Reviews on the depsipeptides have been discussed in several articles including their interesting biological activity (Andavan & Lemmens-Gruber 2010; Maharani *et al*. 2015). Aureobasidin A and petriellin A are two cyclodepsipeptides that contain hydroxy acids in their structures (Figure 1). Aureobasidin A has 2-hydroxy-2-methylpentanoic acid (Hmp), while petriellin A has phenyllactic (Lee et al., 1995; Takesako et al., 1991). Besides Hmp and penyllactic acid, other α-hydroxy acids are also found in nature, such as Hiv in aureobasidin B (Figure 1) and also leucic acid in exumolide A and B (Jenkins *et al*. 1998; Takesako *et al*. 1991).

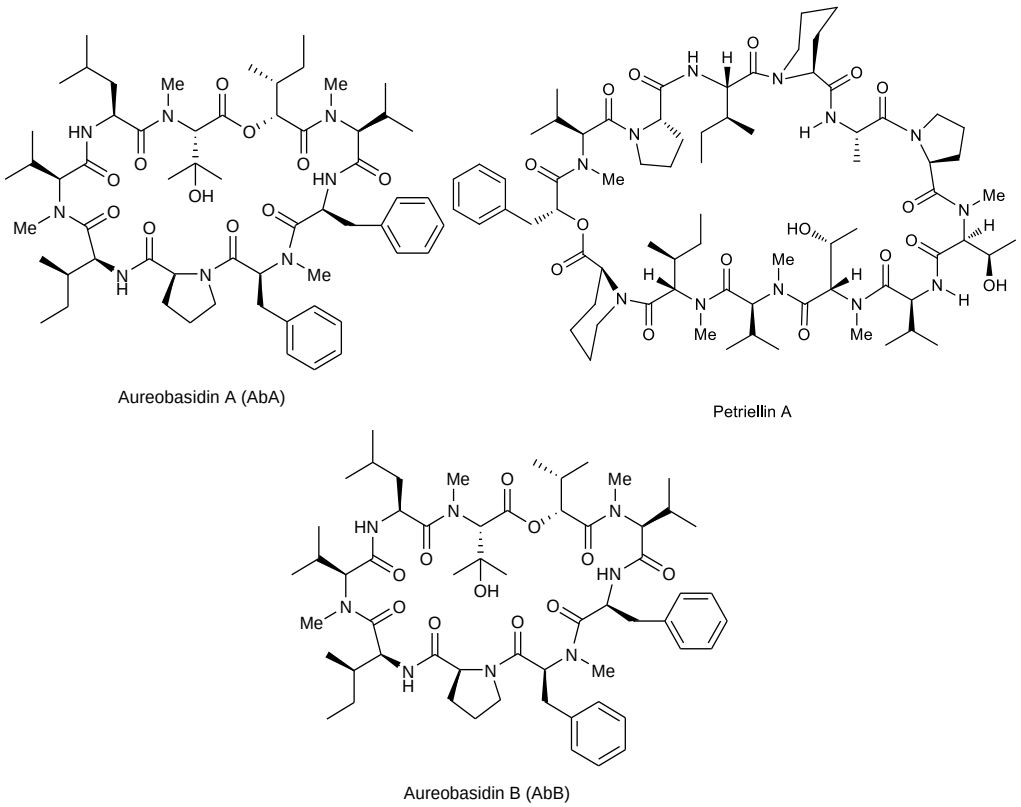
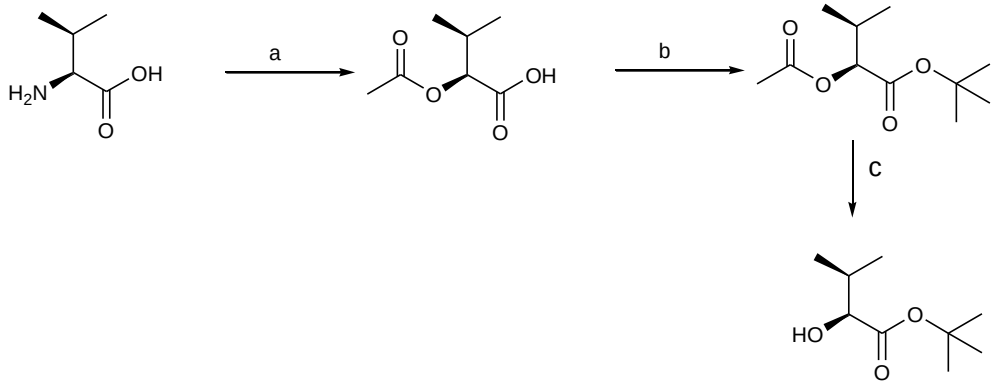


Figure 1. Structure of aureobasidin A (AbA), petriellin A, and aureobasidin B (AbB).



Scheme 1. Synthesis of Hiv-*t*-Bu, (a) acetic acid, $NaNO_2$; (b) *t*-butanol, $(Boc)_2O$, DMAP; (c) K_2CO_3 in 50% MeOH in water (Maharani *et al*. 2017)

In this present work, we reported a preparation of Hiv as a residue of AbB. Maharani *et al*. (2017) has successfully synthesized Hiv-t-Bu (Scheme 1) based on similar synthesis of hydroxy acids by Sleebs *et al*. (2011) and Maharani *et al*. (2014). Hiv was synthesized as its acetyloxy and was then *t*-Bu-protected at its carboxyl moiety to give fully protected Hiv (Ac-Hiv-*t*-Bu). The acetyloxy group of this compound was then deprotected giving Hiv-*t*-Bu. This precursor will be used for the coupling with the Fmoc-β-OH-MeVal giving Fmoc-depsipeptide as a precursor of AbB in solid-phase synthesis of AbB. The later plan has not been realized and being a plan of our future synthetic target.

Currently, an alternative way to synthesize AbB was designed. Hiv was planned to be attached on resin not as its Fmoc-depsidipeptide but as unprotected Hiv. This plan followed a strategy used by Coin *et al*. (2007) in the synthesis of cotransin where lactic acid was directly attached on the resin. Hiv was synthesized using protocols described in Zubia *et al*. (2005) and Cohen-Arazi *et al*. (2008). Amino acid was diazotized using $NaNO_2$ in sulfuric acid as catalyst. The two protocols have differences particularly in the ratio of $NaNO_2$ to amino acid employed in the synthesis. Zubia *et al*. (2005) used 3:1 ratio, while Cohen-Arazi *et al*. (2008) employed 6:1 ratio. In the present paper, a ratio of 3:1; 4:1; 5:1;

and 6:1 between $NaNO_2$ and amino acid were trialed and investigated to find the best condition giving the highest yield of hydroxy acid. Further, the best result would be applied for the synthesis of Ac-Hiv as the precursor of Fmoc-depsidipeptide of AbB if it could increase the prior 63% yield, reported by Maharani *et al*. (2017).

MATERIALS AND METHODS

General procedure

D-valine was purchased from GL-Biochem, Shanghai, China. Infrared spectrum was obtained in FT-IR Perkin Elmer instrument. ^{1}H- and ^{13}C-NMR were measured in NMR Agilent 500 MHz (^{1}H) and 125 MHz (^{13}C) using deuterated solvent. Reagents used were in analytical grade for synthesis. Reaction was monitored by thin layer chromatography using silica gel plate GF_{254} with UV detector at λ 254 nm and 365 nm.

Formation of (2R)-hydroxyisovaleric acid

Synthesis of Hiv was carried out using modified protocols of Zubia *et al*. (2005) and Cohen-Arazi *et al*. (2008).

D-valine (2 g, 17 mmol) was dissolved in 1 M sulfuric acid (30 mL) and sodium nitrite (3, 4, 5, and 6 eq.) was dissolved in water (30 mL). Sodium nitrite solution was added drop wisely at 0°C for 2 hours. Reaction mixture was then stirred at room temperature for 17 hours. the reaction was monitored by thin layer chromatography (TLC) with eluent of propanol:methanol:acetic acid (7:3:1), and then, the TLC plate was sprayed with ninhydrin solution in acetone. After the reaction was finished, the reaction mixture was added with sodium bicarbonate until pH 2 and then added by sodium chloride until the mixture was saturated. The reaction mixture was filtered and the filtrate was extracted with ethyl acetate (3 × 50 mL). Ethyl acetate fraction was then dried with sodium sulfate and filtered. The organic solution was then evaporated and freeze dried to give yellowish solid for 1.27 g (63.3 %). The purity was analysed by TLC and characterized by spectroscopic UV, IR, and ^{1}H-NMR methods. Hiv: rf value 0.8 (propanol:metanol:acetic acid 7:3:1); ^{1}H-NMR (500 MHz, CD_3OD) δ 0.99 (d, J=6.9 Hz, 3H), 0.99 (d, *J*=7 Hz, 3H), 2.44 (m, 1H), 3.94 (d, *J*=4.2, 1H), 5.29 (s, 1H).

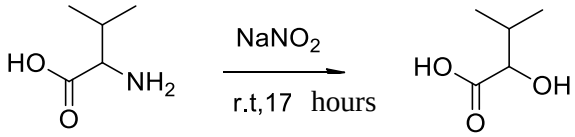
Formation of (2*R*)-acetyloxyisovaleric acid

Methodology for the synthesis of (2*R*)-acetyloxyisovalerate (Ac-Hiv) was carried out through a methodology applied for the synthesis of *t*-butyl (2*S*)-hydroxy-(3*S*)-methylpentanoate and Ac-Hiv (Maharani *et al*. 2014; Maharani *et al*. 2017).

D-valine (1.5 g;17 mmol) was dissolved in glacial acetic acid (25 mL). The stirred solution was cooled occasionally to keep the solution at room temperature. To this solution was added sodium nitrite (7 g;102 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 ethereal 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 for 1.426 g (71.3%) as a colourless oil. Ac-Hiv: rf value 0.7 (propanol:methanol 7:3). ^{1}H NMR (500 MHz, CD_3OD) δH (ppm) 5.45 (s, 1H), 4.79 (d, *J* = 4.3 Hz, 1H), 2.22 (dtd, *J* = 13.8, 6.9, 4.4 Hz, 1H), 2.11 (s, 3H), 1.01 (dd, *J* = 12.0, 6.9 Hz, 7H). ^{13}C NMR (126 MHz, CD_3OD) δC (ppm) 171.89, 171.22, 76.97, 29.71, 19.29, 17.85, 16.23.

RESULTS AND DISCUSSION

Preparation of Hiv was initiated by dissolving D-valine in aqueous sulfuric acid before it was added by aqueous $NaNO_2$ at 0°C for 2 hours and finally stirred for 17 hours at room temperature (Scheme 2). The reaction was monitored by thin layer chromatography using eluent of propanol:methanol:acetic acid (7:3:1). The TLC plate was then sprayed by ninhydrin reagent and heated to see if the amino acid has been disappeared in the reaction mixture or not. The presence of purple colour on the TLC plate showed that the amino acid has not been fully converted to the hydroxy acid.



Scheme 2. Synthesis of Hiv (aq. H_2SO_4, added by $NaNO_2$ at 0°C for 2h, stirred at rt for 17 hours).

The ratios of $NaNO_2$ to amino acid was trialed in several experiments (3:1; 4:1; 5:1; and 6:1). It was found that 6:1 ratio of $NaNO_2$ to amino acid could give the best result with 63% yield. The reaction was monitored by thin layer chromatography after 17 hours stirring at room temperature. After 17 hours, the reaction mixture with 6:1 ratio gave the absence of purple colour on the TLC plate after sprayed with ninhydrin reagent and heated. Meanwhile, other ratios showed that the conversion of amino acid into hydroxy acid has not completed after 17 hours.

Hiv was characterized by using spectroscopic methods. Ultraviolet spectrum (Figure 2) showed an absorption on wavelength of 206 nm (ε = 132.8 nm), indicating the presence of transition of n to π* from the carbonyl group.

The infrared spectrum (Figure 3) showed vibrational stretches of O-H at 3425 cm^{-1}, sp^3 C-H at 2971 cm^{-1}, and carboxylic acid C=O, at 1722.1 cm^{-1} that are characteristics for the Hiv.

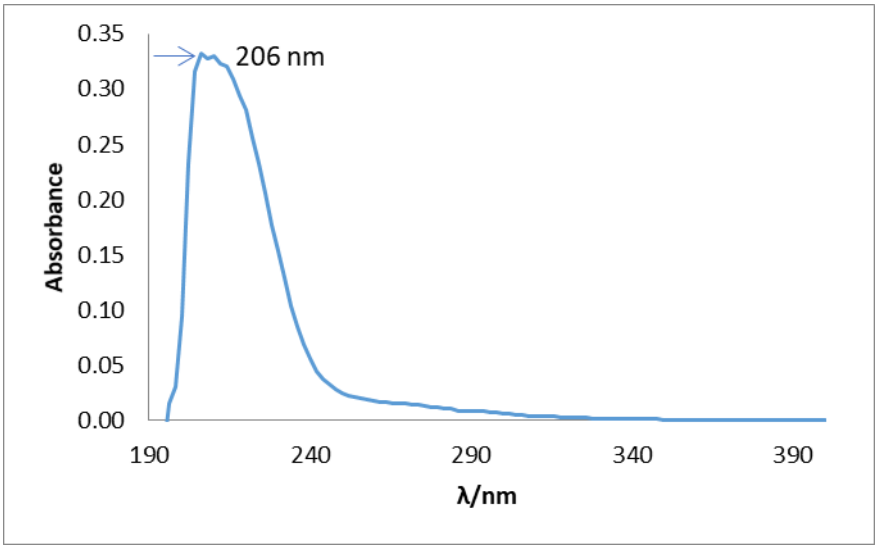


Figure 2. Ultraviolet spectrum of Hiv in methanol.

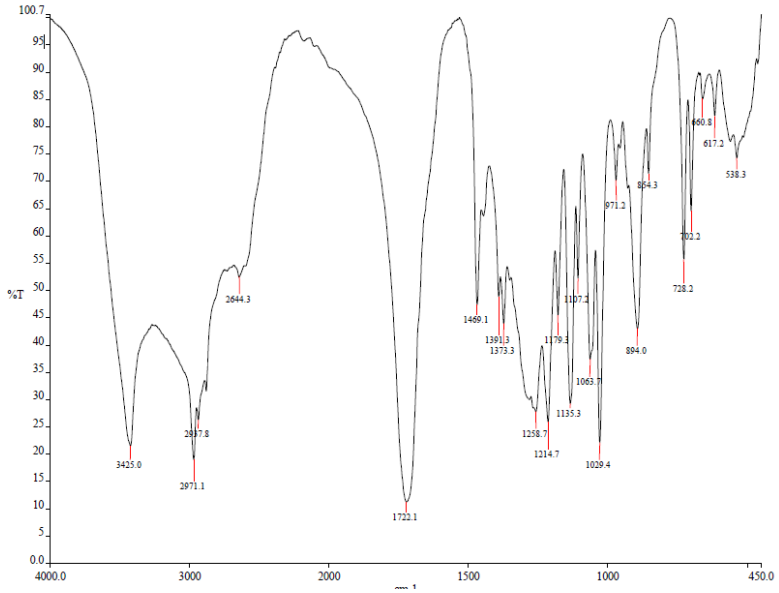
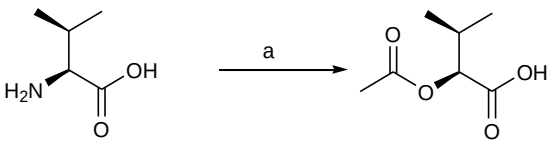


Figure 3. Infrared spectrum of Hiv in KBr plate

^{1}H-NMR spectra of Hiv in CD_3OD (Figure 4) showed a doublet signal of three protons at 0.91 ppm (*J*=6.9 Hz), representing methyl proton, coupled to one methine proton. Another signal of three protons emerged at chemical shift of 1.01 ppm (d, *J*=7 Hz), indicating the second methyl proton that has methine proton as its neighbour. A chemical shift for multiplet proton at 2.08 ppm represented methine proton, coupled with six protons of two methyls. Proton of the oxygenated methine was emerged at chemical shift of 3.95 ppm (d, *J* = 4.2 Hz). Meanwhile, two proton signals at 5.30 ppm belongs to two hydroxyl groups from the Hiv.

Conversion of D-Val (Scheme 3) into its α-acetyloxy acid was carried out using a protocol described by Maharani *et al*. (2017). The diazotisation reaction was carried out in the presence of glacial acetic acid/$NaNO_2$ generating 71% yield of the desired product (Scheme 3).



Scheme 3. Synthesis of (2*R*)-acetyloxyisovaleric acid, (a) acetic acid, $NaNO_2$.

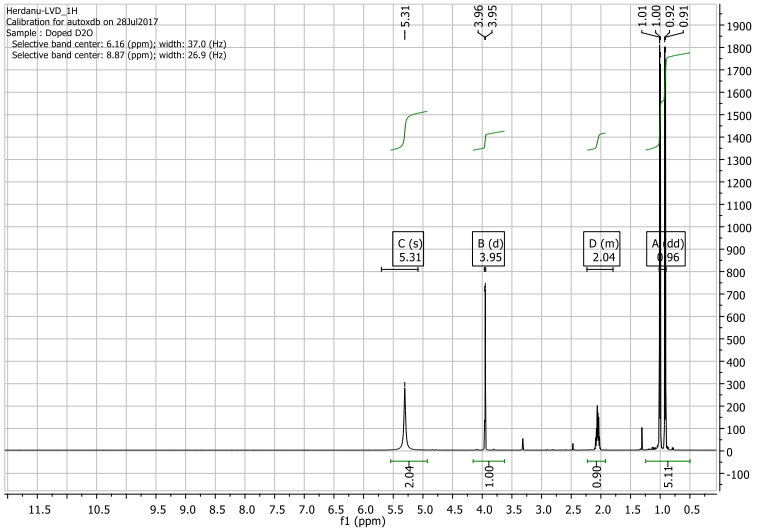


Figure 4. ^{1}H-NMR spectrum of Hiv in CD_3OD (Agilent, 500 MHz).

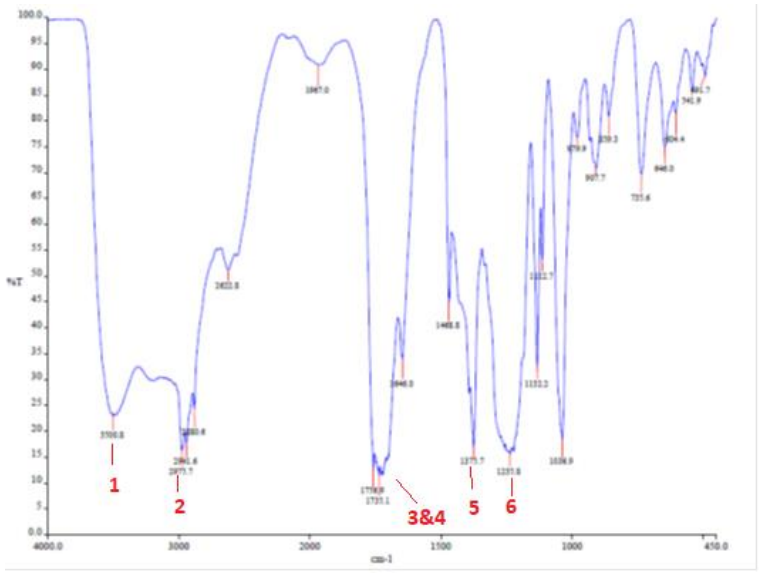


Figure 5. Infrared spectrum of Ac-Hiv in NaCl pellet.

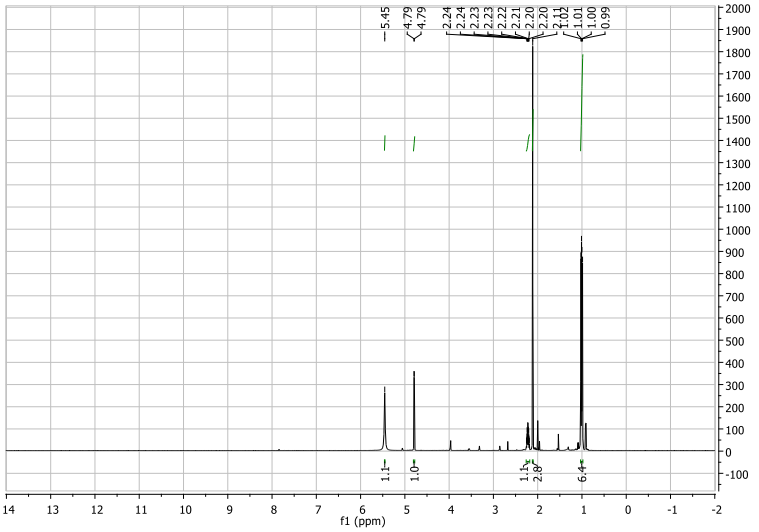


Figure 6. ^{1}H-NMR spectrum of Ac-Hiv in CD_3OD (Agilent, 500 MHz).

The yield was found to be higher than the previously reported article (63% yield) (Maharani *et al*. 2017) due to change of the ratio between amino acid and sodium nitrite. The good result in the synthesis of Hiv with 1:6 ratio between amino acid and sodium nitrite was applied for the synthesis of Ac-Hiv that priorly used 1:3 ratio.

Product of synthesis was characterized by IR and ^{1}H-NMR. IR analysis showed the presence of broad O-H stretch with υ_{max} 3300 cm^{-1} that is typical for –COOH group (Figure 5). Two C=O stretches at υ_{max} 1756 and 1735 cm^{-1} showed the presence of two carbonyl groups of respective ester and carboxylic groups. The presence of a signal at δH 2.15 ppm in the ^{1}H-NMR represented methyl protons in the acetate moiety (Figure 6). In addition, the presence of one additional carbonyl signal (δC 171.4 ppm) showed that the acetate was present in the structure.

CONCLUSIONS

Hiv was synthesised through diazotisation of D-valine with a ratio of 1:6 (amino acid: sodium nitrite) giving the best yield (70% yield) after 17 h reaction. The increase of the equivalent of sodium nitrite (6 eq) was applied in the synthesis of Ac-Hiv showing the increase of the yield from 63% to 71% yield.

ACKNOWLEDGMENT

RM and all co-authors would like to thank Kemenristek-DIKTI (PDUPT grant) for financial support.

REFERENCES

Andavan, G. S. B., & Lemmens-Gruber, R. (2010). Cyclodepsipeptides from marine sponges: Natural agents for drug research. *Marine Drugs.* 8(3): 810-834.

Cohen-Arazi, N., Katzhendler, J., Kolitz, M., & Domb, A. J. (2008). Preparation of new α-hydroxy acids derived from amino acids and their corresponding polyesters. *Macromolecules.* 41(20): 7259-7263.

Coin, I., Beyermann, M., & Bienert, M. (2007). Solid-phase peptide synthesis: from standard procedures to the synthesis of difficult sequences. *Nature Protocols*. 2(12): 3247-3256.

Jenkins, K. M., Renner, M. K., Jensen, P. R., & Fenical, W. (1998). Exumolides A and B: Antimicroalgal cyclic depsipeptides produced by a marine fungus of the genus Scytalidium. *Tetrahedron Letters*. 39(17): 2463-2466.

Lee, K. K., Gloer, J. B., Scott, J. A., & Malloch, D. (1995). Petriellin A: a novel antifungal depsipeptide from the coprophilous fungus *Petriella sordida*. *The Journal of Organic Chemistry*. 60(17): 5384-5385.

Luesch, H., Williams, P. G., Yoshida, W. Y., Moore, R. E., & Paul, V. J. (2002). Ulongamides A− F, new β-amino acid-containing cyclodepsipeptides from palauan collections of the marine *Cyanobacterium lyngbya* sp. *Journal of Natural Products*. 65(7): 996-1000.

Maharani, R., Brownlee, R.T., Hughes, A.B., & Abbott, B.M. (2014). A total synthesis of a highly *N*-methylated cyclodepsipeptide [2*S*, 3*S*-Hmp]-aureobasidin L using solid-phase methods. *Tetrahedron*. 70(14): 2351-2358.

Maharani, R., Puspitasari, D., Huspa, D. H. P., Hidayat, A. T., Sumiarsa, D. & Hidayat, I. W. (2017). Synthesis of *t*-butyl (2*R*)-hydroxyisovalerate, a precursor of aureobasidin B. In IOP Conference Series: Materials Science and Engineering. 172(1): 012044.

Maharani, R., Sleebs, B. E., & Hughes, A. B. (2015). Macrocyclic *N*-methylated cyclic peptides and depsipeptides. *Studies in Natural Products Chemistry*. 44: 113-249.

Sasaki, T., Takagi, M., Yaguchi, T., Miyadoh, S., Okada, T., & Koyama, M. (1992). A new anthelmintic cyclodepsipeptide, PF1022A. *The Journal of Antibiotics*. 45(5), 692-697.

Sleebs, M.M., Scanlon, D., Karas, J., Maharani, R., & Hughes, A.B. (2011). Total synthesis of the antifungal depsipeptide petriellin A. *The Journal of Organic Chemistry*. 76(16): 6686-6693.

Suzuki, A., Taguchi, H., & Tamura, S. (1970). Isolation and structure elucidation of three new insecticidal cyclodepsipeptides, destruxins C and D and desmethyldestruxin B, produced by *Metarrhizium anisopliae*. *Agricultural and Biological Chemistry*. 34(5): 813-816.

Takesako, K., Ikai, K., Haruna, F., Endo, M., Shimanaka, K., Sono, E., Yamaguchi, H. (1991). Aureobasidins, new antifungal antibiotics taxonomy, fermentation, isolation, and properties. *The Journal of Antibiotics*. 44(9): 919-924.

Tomoda, H., Nishida, H., Huang, X.-H., Masuma, R., Kim, Y. K., & Omura, S. (1992). New cyclodepsipeptides, enniatins D, E and F produced by *Fusarium sp*. FO-1305. *The Journal of Antibiotics*. 45(8): 1207-1215.

Zubia, A., Mendoza, L., Vivanco, S., Aldaba, E., Carrascal, T., Lecea, B., Cossío, F. P. (2005). Application of stereocontrolled stepwise [3+ 2] cycloadditions to the preparation of inhibitors of $\alpha_4\beta_1$-integrin-mediated hepatic melanoma metastasis. *Angewandte Chemie*. 117(19): 2963-2967.