Jurnal Kimia VALENSI: Jurnal Penelitian dan Pengembangan Ilmu Kimia, 2(2), November 2016, 85-90

Available online at Website: http://journal.uinjkt.ac.id/index.php/valensi

Solid-phase Synthesis of Tetrapeptide on 2-Chlorotrityl Chloride Resin by Using Benzotriazol-1-yl-oxytripyrrolidinophosphonium Hexafluorophosphate as Coupling Reagent

Rani Maharani, Nuruzzahra Ammatillah, Gunawan, Ace Tatang Hidayat

Department of Chemistry Jalan Raya Bandung-Sumedang Km 21 Jatinangor Kabupaten Sumedang 45363 West Java Indonesia

Email: r.maharani@unpad.ac.id

Received: October 2016; Revised: November 2016; Accepted: November 2016; Available Online: December 2016

Abstract

Tetrapeptide, OH-Pro-Ala-Gly-Tyr-NH_2, was successfully synthesised on 2-chlorotrityl chloride resin by taking advantage of PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) as coupling reagent. The selection of the peptide as the target of synthesis was due to its interesting bioactivity as antioxidant. The synthesis was undertaken with Fmoc strategy, where Fmoc-proline was added onto the resin at the first place. It is known from the literature that proline can resist from rasemisation when it was attached on the resin at the first time. Fmoc deprotection step was carried out by employing 20% piperidine in DMF and the reaction mixture was shaken for 30 minutes. Once the proline attached, the next step was to attach amino acids, alanine (Ala), glycine (Gly) and tyrosine(Tyr), subsequently onto the resin until tetrapeptidyl resin was constructed on the resin. Hydroxyl group of Tyr was protected with *t*-butyl, which is TFA-labiled. Coupling reaction was undertaken by mixing the amino acid and PyBOP in a mixture of dichloromethane and DMF (1:1) and in the presence of basic DIPEA. Resin cleavage step was carried out by using 95% TFA in water, where *t*-butyl protecting group on the side chain of Tyr was cleaved at the same time. The analytical RP-HPLC of the final product showed a single peak at 21.9 minutes (20-90% of acetonitrile in water with 0.1% of TFA during 30 minutes), indicating that each coupling has given a good coupling performance and resulting in a pure product. The desired product showed the correct molecular weight with *m/z* 407.2 $[M+H]^+$ and 429.2 [M+Na].

Keywords: *Solid-phase peptide synthesis, tetrapeptide, 2-chlorotrityl chloride, PyBOP, Fmoc strategy.*

DOI: *http://dx.doi.org/10.15408/jkv.v0i0.4055*

1. INTRODUCTION

Peptides are involved in many physiological processes. Their broad acceptance as natural molecules, relatively high stability and well defined actions, have made them attractive for many therapeutic use. Fosgerau and Hoffman (2016) mentioned that peptides can act as hormons, neurotransmitter, growth factors, ion-channel ligands, or anti-infectives.

One of interesting peptides is tetrapeptide, Pro-Ala-Gly-Tyr. This peptide was found to have an antioxidant peptide as Nikoo *et al*., (2014) reported its biological properties after successfully isolated the peptide from gelatine of the skin fish of *Acipenser schrenckii*. Unfortunately,the sequence of the peptide was not clear whether Pro is in the C- or N-terminal. In this current research, we selected OH-Pro-Ala-Gly-Tyr-NH_2 as our target of synthesis in order to test if PyBOP coupling reagent has a good coupling performance in the synthesis of the tetrapeptide.

Solid-phase peptide synthesis was chosen over solution-phase synthesis due to some reasons. Total synthesis of some peptides in solution phase has been clearly shown to be challenging and time consuming (Pettit *et al.,* 1986; Rinehart *et al.,* 1987; Ewing *et al.,*1989; Bates *et al.,*1997; Wenger, 1983; Nakamura *et al.,*1995; Mutou *et al.,*1996). Most of the time, purification between steps is also required which also impacts the yield. Solid-phase

P-ISSN: 2460-6065, E-ISSN: 2548-3013

peptide synthesis, pioneered by Merrifield, is a useful method to overcome some of the problems inherent in solution chemistry and has been applied to successfully synthesise several peptides (Thern *et al.,* 2002; Sleebs *et al.,* 2011).

In order to develop a new efficient method to synthesise the tetrapeptide OH-Pro-Ala-Gly-Tyr-NH_2, in this current report, we reported the total synthesis of OH-Pro-Ala-Gly-Tyr-NH_2 with solid-phase method on 2-chlorotrityl chloride resin. The synthesis was based on Fmoc strategy. PyBOP (benzotriazol-1-yl-oxytripyrrolidino phosphonium hexa fluoro phosphate) coupling reagent was selected in the peptidic bond formation. This selection was due to the fact that PyBOP is efficient and could generate a less toxic by-products (Montalbetti and Falque, 2005). Once all of the amino acids were attached on the resin, the resin cleavage took advantage of 95% of TFA in water.

2. MATERIAL AND METHOD

All of the residues were purchased from GL-Biochem Ltd., China. All of amino acids are L-configured. After all of the residues were ready, the next step was solid-phase peptide synthesis of the linear tetrapeptide that will be explained as follows. The synthesis was based on the protocol described by Maharani *et al*. (2014).

Resin Loading

To 2-chlorotrityl resin (0.45 g g, 0.5mmol) was added dry dichloromethane (5 mL) and a solution of Fmoc-L-proline (211 mg, 0.63 mmol) that was previously treated with dry dichloromethane (10 mL) and *N,N*-diisopropylethylamine (0.43 mL, 2.5 mmol). The mixture was shaken for 3-4 h. Methanol (2 mL) was then added and the mixture was shaken for another 10 min. The latter step was undertaken in two cycles. The resin was then filtered and washed successively with dichloromethane, dimethylformamide and dichloromethane. The resin was dried *in vacuo* for 30 min by a stream of air to obtain dry Fmoc-propylresin. A 0.6 mmol portion of dry Fmoc-propyl resin was employed in the synthesis of the tetrapeptide.

Fmoc Deprotection

Fmoc-peptidyl resin was shaken with 20% dry piperidine in dry dimethylformamide (10 mL) for 30 min. The resin was then filtered and washed with dichloromethane, dimethylformamide and dichloromethane successively. The exposed primary amine was analysed by mass spectrofotometry and thin layer chromatography.

PyBOP–mediated Coupling

To the dry peptidyl resin was added the Fmoc-amino acid (1.2 mmol) that was previously treated with benzotriazol-1-yl-oxytripyrrolidino phosphonium hexa fluoro phosphate (640 mg, 1.2 mmol) and *N,N*-diisopropylethyl amine (640 µL, 3.8 mmol) in dry dichloromethane:dimethylformamide (1:1, 5 mL) the resin was shaken for 24 h. the resin was filtered and washed with dichloromethane, dimethylformamide and dichloromethane successively.

Resin Cleavage

To the peptidyl-trichlorotrityl resin (1.2 mmol) was added a cleavage cocktail of trifluoroaceticacid:dry dichloromethane (1:19, 10 mL). The yellow resin turned bright red. The resin was then shaken for 1 h and then filtered. The resin was washed subsequently with further cleavage cocktail (10 mL x 2) and dry dichloromethane (10 mL x 2). The combined solution was evaporated and the resulting residue was dissolved in acetonitrile:water (1:1, 10 mL). The solution was then freeze-dried to obtain a brown solid (1.30 g). The crude solution (20 µL, 1 mg/1mL in 50% acetonitrile in water) was subjected to analytical RP-HPLC on Apollo 5µ, C18 column (250 mm × 4.6 mm), 20%-90% acetonitrile in water over 20 min, flow rate 1 mL/min, 40 ^{o}C, and monitored at 240 nm.

3. RESULTS AND DISCUSSIONS

Synthesis of OH-Pro-Ala-Gly-Tyr-NH_2 was started by attaching the first amino acid, Fmoc-L-proline, onto the 2-chlorotrityl resin (Figure 1). This step was undertaken in dichloromethane in the presence of a base, DIPEA. The mixture was shaken for 24 hours to facilitate complete attachment. The resin was found to have an amino acid loading of 0.52 mmol/ 1.00 g resin.

Truncated products were avoided by adding methanol to cap any of the remaining chloro groups on the resin (Figure 2). This capping would ensure that there was only the amino group of the proline available for the next coupling.

The next step was to remove Fmoc protecting group from the Fmoc-prolylresin, which took advantage of 20% piperidine in DMF, where the reaction mixture was shaken for 30 minutes (Figure 3). After washing the resin with DMF and dichloromethane, the dry resin was tested before it was ready for coupling with a second amino acid. A few beads of the dry resin were tested with a chloranil test with a positive result suggesting the presence of a secondary amino acid due to complete removal of the Fmoc protecting group.

Couplings of the second amino acids, Fmoc-L-Ala, was carried out by using PyBOP coupling reagent, (Figure 4). The coupling system was found to give a complete attachment of the second residues after 24 hours coupling. The MS analysis was applied to see the coupling effectiveness. The successful deprotection was also tested by TLC analysis, where the absence of the Fmoc group was shown by the absence of the spot on the TLC plate.

The following couplings for respective Fmoc-Gly and Fmoc-Tyr(O-*t*Bu) employed the same coupling reagent PyBOP. Repetitive protocols, Fmoc deprotection and coupling, were carried out until the desired tetrapeptide was obtained. The Fmoc-tetrapeptidyl resin was analysed by ESI-MS showing the correct molecular ion of *m/z* 629.4 ($M+H^+$) and *m/z* 651.3 (M+Na) (Figure 5). After the confirmation of the presence of the protected peptide on resin, the next step was to deprotect the Fmoc group, so that the desired unprotected tetrapeptide was ready to be cleaved from the resin.

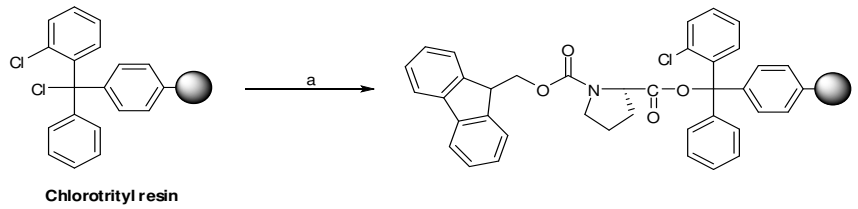

Figure1. Attachment of the first amino acid, (a) Fmoc-L-Pro, CH_2Cl_2, DIPEA.

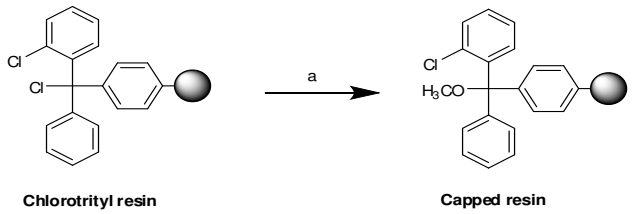

Figure2. Capping the resin, (a) methanol.

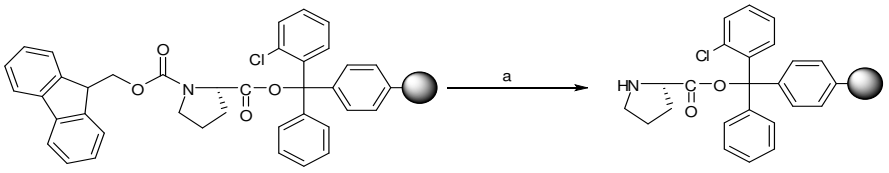

Figure 3. Fmoc removal, (a) 25% piperidine in DMF, 30 minutes.

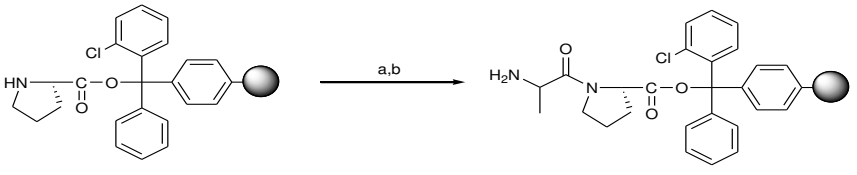

Figure 4. Second residue attachment, (a) Fmoc-L-Ala, (b) 20% piperidine in DMF.

The tetrapeptidyl resin of OH-Pro-Ala-Gly-Tyr-NH_2 was cleaved from the resin by the treatment of the peptide with a cocktail of 95% TFA in water and the reaction mixture was shaken for one hour resulting in the cleaved tetrapeptide (Figure 6). Water was added into the cocktail due to its scavenging properties for the reactive t-butyl species that was eliminated during the resin cleavage step. The yellow beads resin turned red during the addition of the TFA cocktail, indicating that the peptideshad been cleaved from the resin. The resin was washed with dichloromethane several times with the cleavage cocktail and the washings were combined. The solvent was then removed by rotary evaporation at room temperature. This step was undertaken several times in order to remove any trace of TFA.

The peptide crude was analysed by ESI-MS, showing a correct molecular weight with *m/z* 407.2 $[M+H]^+$ and 429.2 [M+Na]. The ESI-MS spectrum can be seen in Figure 7. Spectrum of analytical RP-HPLC of the crude (Figure 8) showed that the crude has a high purity with a good and clean HPLC spectrum. The desired linear peptide was identified, with a retention time of 21.9 minutes.

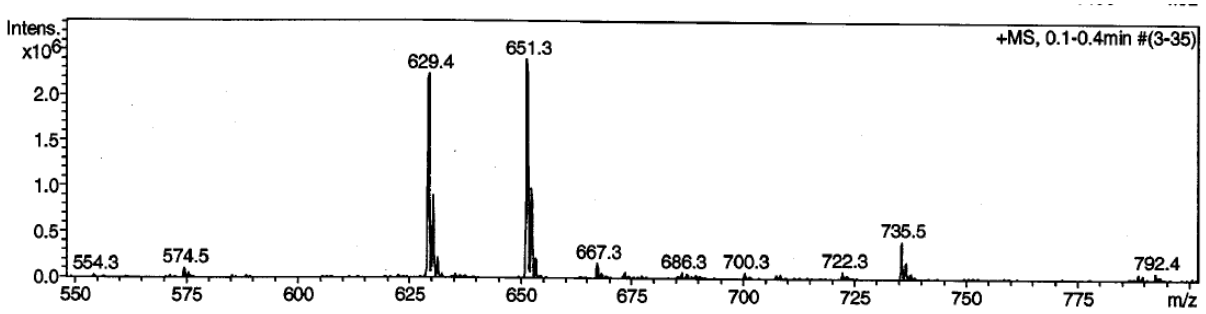

Figure 5. ESI-MS spectrum of linear tetrapeptide, OH-Pro-Ala-Gly-Tyr-NH_2.

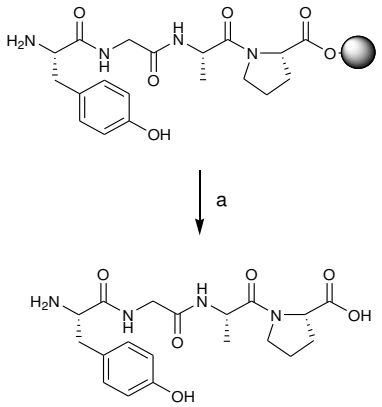

Figure 6. Resin cleavage step of tetrapeptidyl resin, (a) 95% TFA in water.

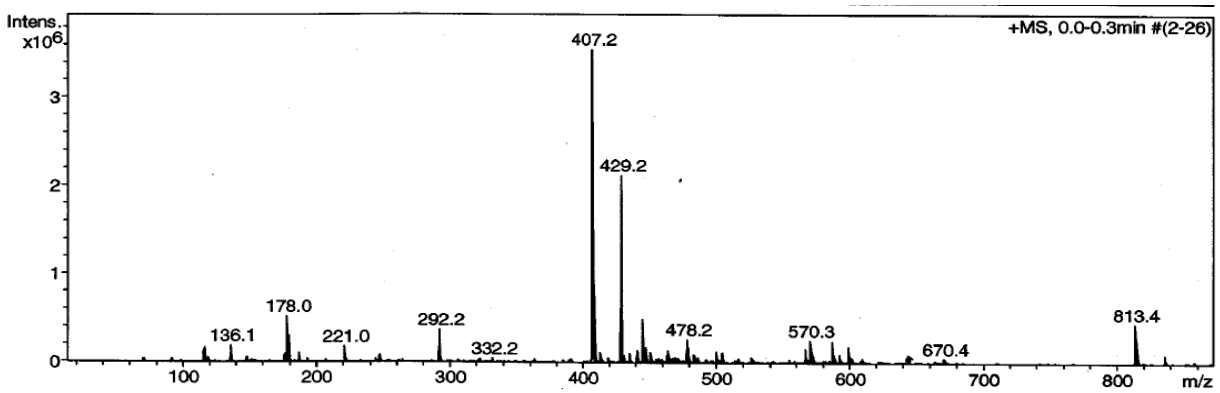

Figure 7. ESI-MS spectrum of linear tetrapeptide, OH-Pro-Ala-Gly-Tyr-NH_2.

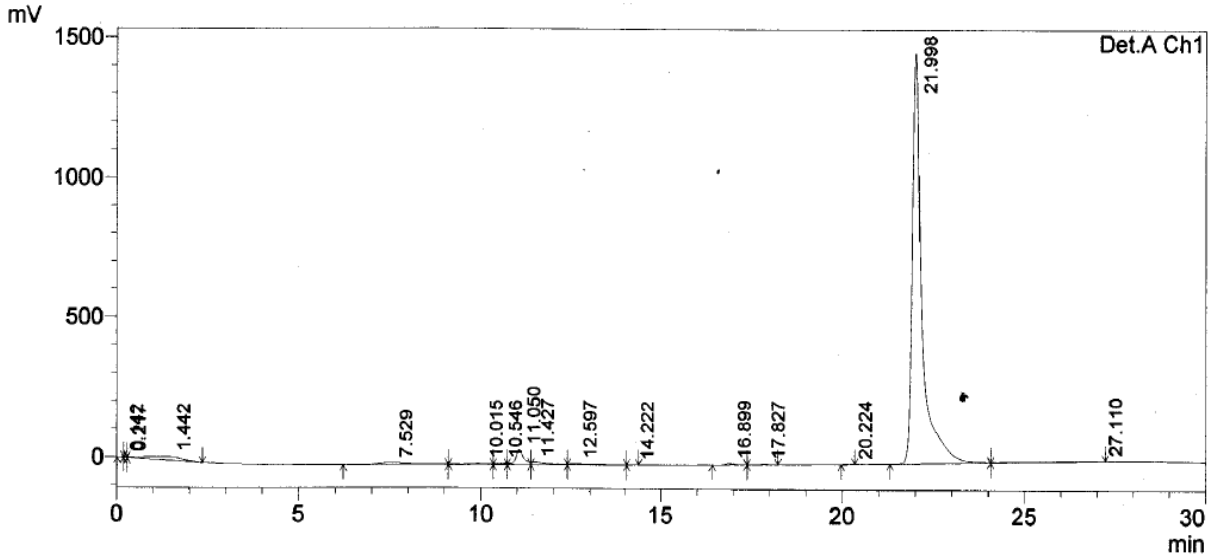

Figure 8. Analytical RP-HPLC spectrum of tetrapeptide crude (acetonitrile:water in a presence of 0.1% TFA, 20-90% over 30 min).

Structure of the final product can be seen in Figure 9.

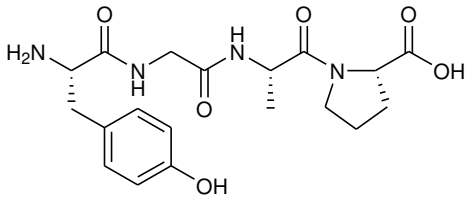

Figure 9. Structure of OH-Pro-Ala-Gly-Tyr-NH_2.

4. CONCLUSION

A linear tetrapeptide, OH-Pro-Ala-Gly-Tyr-NH_2 has been synthesised successfully through a solid-phase peptide synthesis method by using PyBOP as coupling reagent. Linear peptidewas analysed by analytical RP-HPLC and ESI-MS. The desired product showed the correct molecular weight with *m/z* 407.2 $[M+H]^+$ and 429.2 [M+Na].

ACKNOWLEDGMENT

RM would like to thank KLN-DIKTI research grant for financial support of the research.

REFERENCES

Bates RB, Brusoe KG, Burns JJ, Caldera S, Cui W, Gangwar S, Gramme MR, McClure KJ, Gregory P, Schadow H Dolastatins. 1997. Synthesis and stereochemistry of dolastatin 11[1a]. *J. Am. Chem. Soc.* 119(9): 2111-2113.

Ewing WR, Harris BD, Li WR, Joullie MM. 1089. Synthetic studies of didemnins IV. Synthesis of the macrocycle. *Tetrahedron Lett.* 30(29): 3757-3760.

Fosgerau K, Hoffmann T. 2015. Peptide therapeutics: current status and future directions. *Drug discovery today*. 20(1): 122-128.

Maharani R, Brownlee RT, Hughes AB, Abbott BM. 2014. A total synthesis of a highly N-methylated cyclodepsipeptide [2S,3S-Hmp]-aureobasidin L using solid-phase methods. Tetrahedron. 70:2351-1358.

Merrifield RB. 1963. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* 85(14): 2149-2154.

Montalbetti CA, Falque V. 2005. Amide bond formation and peptide coupling. *Tetrahedron*. 61: 10827-10852.

Mutou T, Kondo T, Shibata T, Ojika M, Kigoshi H, Yamada K. 1996. Synthesis of dolastatin G and nordolastatin G, cytotoxic 35-membered cyclodepsipeptides of marine origin. *Tetrahedron Lett.* 37: 7299-7302.

Nakamura M, Shibata T, Nakane K, Nemoto T, Ojika M, Yamada K. 1995. Stereochemistry and total synthesis of

dolastatin E. *Tetrahedron Lett.* 36: 5059-5062.

Nikoo M, Benjakul S, Ehsani A, Li J, Wu F, Yang N, Xu B, Jin Z, Xu X. 2014. Antioxidant and cryoprotective effects of a tetrapeptide isolated from Amur sturgeon skin gelatin. *Journal of Functional Foods*. 7: 609-620.

Pettit GR, Holzapfel CW. 1986. Structural biochemistry. 25. Antineoplastic agents. 110. Synthesis of the dolastatin 3 isomer cyclo [L-Pro-L-Leu-L-Val-(R, S)-(gln) Thz-(gly) Thz]. *J. Org. Chem.* 51(24): 4580-4585.

Rinehart KL, Kishore V, Nagarajan S, Lake RJ, Gloer JB, Bozich FA, Li KM, Maleczka RE Jr, Todsen WL. 1987. Total synthesis of didemnins A, B, and C. *J. Am. Chem. Soc*. 109: 6846-6848.

Sleebs MM, Scanlon D, Karas J, Maharani R, Hughes AB. 2011. Total Synthesis of the antifungal depsipeptide, Petriellin A. *J. Org. Chem.*

Thern B, Rudolph J, Jung G. 2002. Total synthesis of the nematicidal cyclododecapeptide omphalotin A by using racemization-free triphosgene-mediated couplings in the solid phase. *Angew. Chem., Int. Ed.* 41: 2307-2309.

Thern B, Rudolph J, Jung G. 2002. Triphosgene as highly efficient reagent for the solid-phase coupling of N-alkylated amino acids—total synthesis of cyclosporin O. *Tetrahedron Lett.* 43: 5013-5016.

Wenger RM. 1983. Synthesis of cyclosporine. I. Synthesis of enantiomerically pure (2S,3R, 4R,6E)-3-hydroxy-4-methyl-2-methylamino -6-octenoic acid starting from tartaric acid. *Helv. Chim. Acta*. 66(7): 2308-2321.