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SYNTHESIS OF MONOOLEOYL GLYCEROPHOSPHO HEPTAPEPTIDE AS A CANDIDATE OF PATHOGEN OF ESSENTIAL HYPERTENSION

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Abstract: A monooleoyl glycerophospho heptapeptide was synthesized as a candidate of the endogenous pathogen of essential hypertension. Synthesis of the target compound was achieved by the fragment condensation of the phosphorylated tetrapeptide with a His-containing tripeptide.

A number of isolation studies on the active principle for essential hypertension have been carried out by many groups. Previously, we synthesized DLIS-2, a lysophosphatidylserine as one of the candidate of a hypertensive factor from human plasma.[1] Recently we came across a report by Pang *et al.* describing another active principle of essential hypertension from hypertensive rats. They described its structure as a characteristic heptapeptide **1**, Tyr-Ser-Val-Ser-His-Phe-Arg with monooleoyl glycerophosphatidyl moiety on Ser^4 according to its hydrolytic and enzymatic analysis.[2] Therefore, the synthetic study of this compound seemed to be urgently requested in order to confirm the assumed structure.

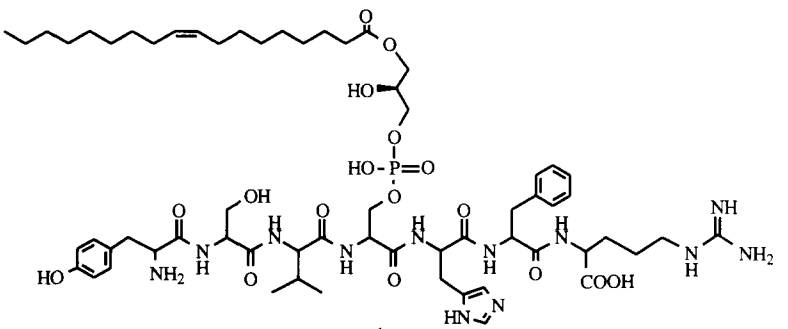

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Fig.1 Assumed structure of a hypertensive factor isolated by Pang *et al.*

In general, simple diacyl glycerophospholipids have been synthesized by the following successive procedures, *i.e.*, the diacylation of glycerol, phosphorylation, and introduction of the base part. Since the target compound in this study is a monooleoyl-glycerophospholipid-peptide conjugate, we set up the synthetic route that the introduction of the oleoyl group should be carried out rather in the final step, because, in that case, the selective protection of 1- and 2-OH group of the glycerol moiety was not necessary, and an easier phosphorylation can be first carried out without a steric hindrance.

First, we tried to synthesize the phosphorylated peptide via the phosphorylation process of a heptapeptide by the phosphite method. The peptide moiety was elongated from *C*-terminus to *N*-terminus by a stepwise method according to the Fmoc strategy[3] using His(Boc) and Arg(Pmc).[4] Two hydroxyl groups on Ser^2 and Ser^4 were selectively protected with Bu^t and Trt groups, respectively. Trt and N^{im}-Boc groups of the heptapeptide thus synthesized were removed with aqueous acetic acid and then N^{im}-Boc group was reintroduced to afford peptide **3** with a free hydroxyl group on Ser^4.

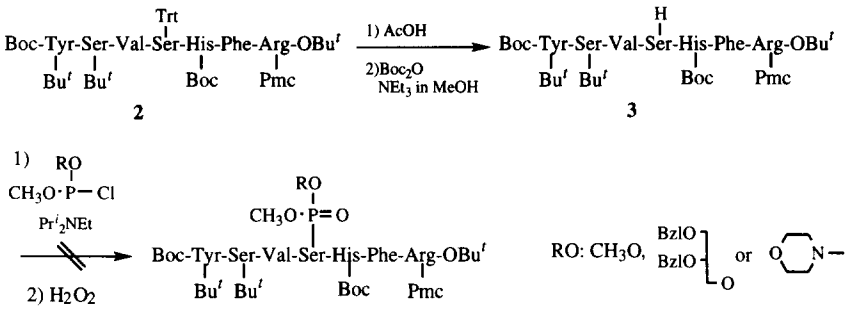

Fig.2 Phosphorylation of heptapeptide **3**

Although phosphitylation of peptide **3** was tried with methyl monochloro morpholidite[5] or other phosphitylating agent, the reaction did not proceed under any conditions as far as applied, presumably due to either a steric hindrance or a participation of imidazole ring in His residue[6]. We turned to adopt a synthetic route through the condensation of a phosphorylated tetrapeptide, prepared from a phospho Ser derivative, with His-containing tripeptide. The synthetic scheme was shown in Fig 3.

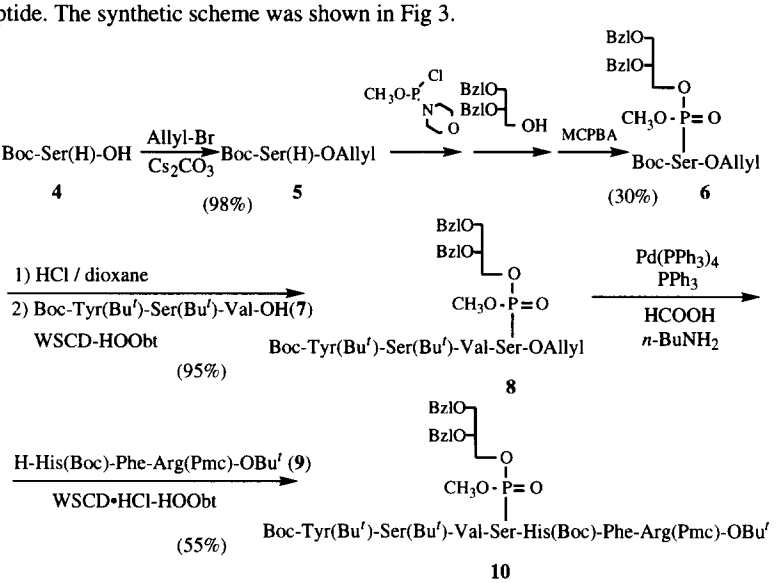

Fig. 3 Alternative synthetic route of phosphorylated heptapeptide **10**

The phosphorylated Ser derivative **6** was prepared through the esterification of Boc-Ser (**4**) with allyl bromide and Cs_2CO_3, followed by glycerophosphorylation using the phosphoramidite method. After deblocking of the Boc group of the Ser derivative, Boc-tripeptide **7** was coupled by WSCD-HOObt method to give the phosphorylated tetrapeptide **8**. After the removal of allyl ester in peptide **8** with Pd(0) complex[7], the peptide was condensed with the *C*-terminal tripeptide **9** to afford the phosphorylated heptapeptide **10** in an excellent yield. This prephosphorylation process afforded the desired heptapeptide in a good yield, so that the reason assumed for the failure of phosphitylation on the His-containing peptide **3** as mentioned above could be plausible and the change of the strategy for preparation of phosphorylated heptapeptide seemed to be reasonable.

In order to introduce an oleoyl group to 1-OH in the glycerol moiety of the phosphorylated peptide, a methyl group of phosphate **10** was deblocked with NaI in 2-butanone (63%) and two Bzl groups were removed

by catalytic hydrogenation (100%) successively as shown in Fig 4. If these deprotection procedures were carried out in the reverse order, no expected compound was afforded due to an elimination of the glycerophosphoryl part through the formation of a cyclic phosphate. Monooleoylation on **11** was performed with oleic acid (2 eq.), WSCD·HCl (2 eq.), and DMAP (0.5 eq.), and then all the protecting groups were deblocked with 95% aq. TFA accompanying the elimination of the phosphatidyl moiety and the addition of TFA to the double bond of the acyl moiety. Thus purification by reverse-phase HPLC gave the desired product **1** in only 1% yield from **11**. However, the compound **1** obtained was identified as the desired oleoyl glycerophosphatidyl heptapeptide **1** by ^{1}H-NMR and plasma-desorption mass spectroscopy (PD-MS). The worse yield may be ascribed to the presence of His residue in close vicinity to phosphoryl group as described above.

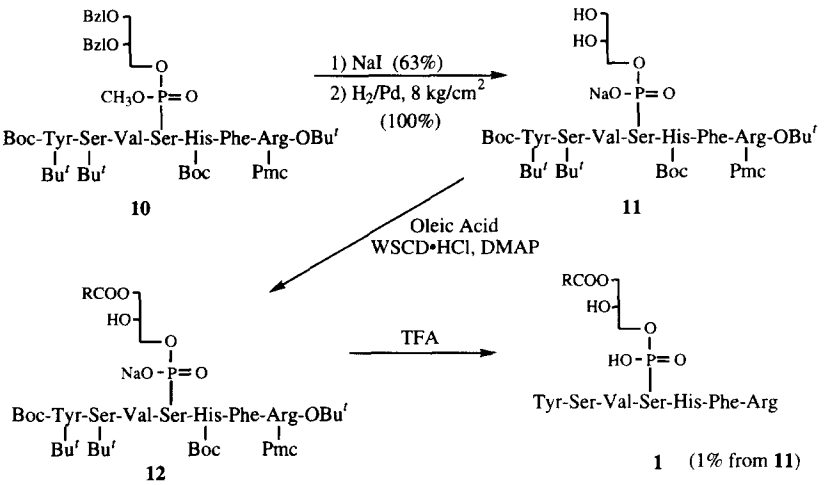

Fig.4 Synthesis of oleoyl glycerophosphatidyl heptapeptide **1**

If one would consider the process of its structure determination by Pang *et al.*, there could not be denied a possibility that the Ser^2-phosphorylated heptapeptide may represent another candidate as an active principle of the essential hypertension.[2,8] We therefore attempted to synthesize the compound bearing the acyl glycerophosphatidyl moiety on Ser^2 residue instead of Ser^4. Trt group was chosen as the protecting groups of the hydroxyl group of both Ser^2 and Ser^4. If the reactivity of the two hydroxyl groups of the Ser residues was distinguishable on the basis of a steric hindrance as well as a neighboring His participation, a regioselective phosphorylation at Ser^2 residue would be possible by the use of a limited amount of phosphorylation reagent.

According to the synthetic scheme as depicted in Fig. 5, two Trt and N^{im}-Boc groups of the fully protected heptapeptide **13** were deblocked and then N^{im}-Boc group was reintroduced in the same manner to the case of the Ser^4-phosphorylated peptide **1**. The phosphorylation was performed with morpholidite **15** (5 eq.) to give the desired monophosphorylated peptide **16** as the main product in 11% yield.[9] After a protection by Boc for the non-phosphorylated hydroxyl group to avoid an acylation in the final oleoylation reaction,[10] the protecting groups on both the phosphate and glycerol parts were removed by the successive treatments of NaI and then the catalytic hydrogenation to give compound **17**. Finally, oleoylation was carried out and then the rest of the protecting groups were deblocked with aq. TFA to give the desired compound **19** which was distinguished from the Ser^4-phosphorylated peptide **1** in respect of NMR giving a molecular ion, 1313, on PD-MS.

Although the measurement of the hypertensive activity of the two synthetic peptides, Ser^4- and Ser^2-phosphorylated peptides, are now in progress, the result of their biological activity will judge the structure of the

pathogen of essential hypertension. Moreover, our synthetic method mentioned here provides a novel and general synthetic way for preparation of the phospholipid-peptide conjugate.

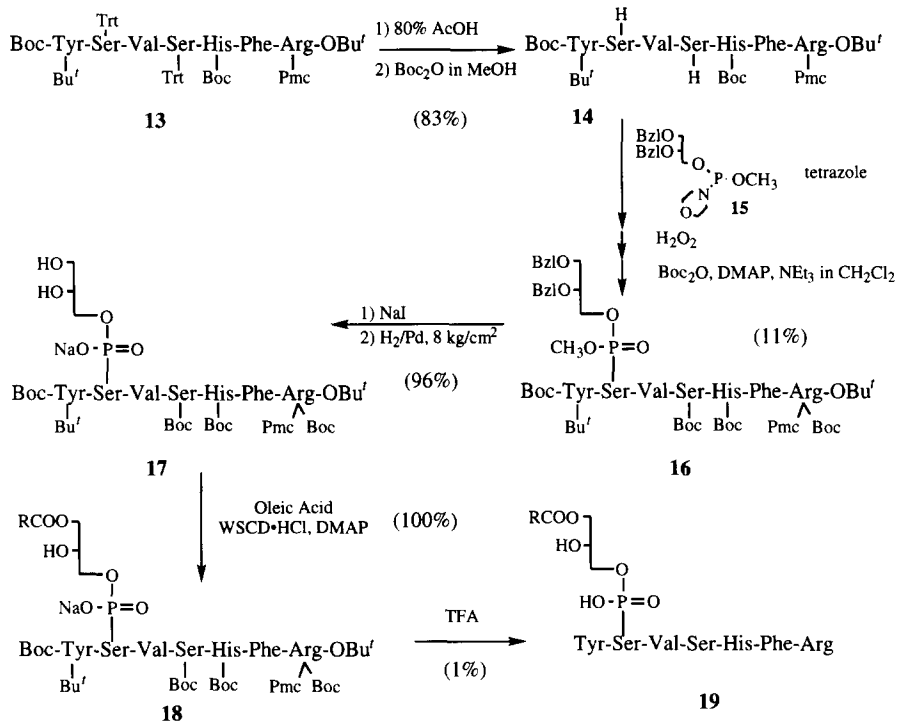

Fig. 5 Synthesis of Ser[2]-phosphorylated heptapeptide **19**

Acknowledgment

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References and Notes

1. Inami, K., Teshima, T., Emura, J., Shiba, T., *Tetrahedron Lett.* **1990**, *31*, 4033.
2. Pang, P. K.T.; Benishin, C. G.; Jie, S.; Lewanczuk, R. Z., *PCT Int. Appl.*, **1993**; WO 9325577.
3. Reviews: Field, G. B., Noble, R. L., *Int. J. Pept. Protein Res.*, **1990**, *35*, 161; Bodanszky, M., *Chemalog Hi-Lites*, **1980**, *4*,5.
4. Abbreviations: Fmoc: 9-fluorenylmethyloxycarbonyl; Boc: *t*-butoxycarbonyl; Pmc: 2,2,5,7,8-pentamethylchroman-6-sulfonyl; WSCD: water-soluble carbodiimide (*N*-dimethylaminopropyl-*N'*-ethylcarbodiimide); HOObt: 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine.
5. Beiter, A. H.; Pfleiderer, W., *Synthesis* **1989**, 497-500.
6. In place of the desired phosphorylation, an elimination of N^{im}-Boc group was observed during the phosphorylation process.
7. Hayakawa, Y.; Kato, H.; Uchiyama, M.; Kajiro, H.; Noyori. R., *J. Org. Chem.* **1986**, *51*, 2402-2404.
8. According to the phosphorylation experiment to the synthetic peptide, a heptapeptide, YSVSHFR, esterified with a monooleoyl glycerophosphate by enzymatic (phospholipase C) method exhibited a hypertensive activity without purification, although the similar phosphorylation product of another heptapeptide, YSVKHFR, showed no activity.
9. Diphosphoryl peptide was not detected during this reaction.
10. A di-Boc product was obtained. Another Boc group was supposed to be introduced to the guanidino group on Arg residue.

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