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SOLID-PHASE SYNTHESIS OF PEPTIDES CONTAINING *O*-PHOSPHORYL SERINE AND *O*-PHOSPHORYL THREONINE USING ALLYL GROUP FOR PHOSPHATE PROTECTION

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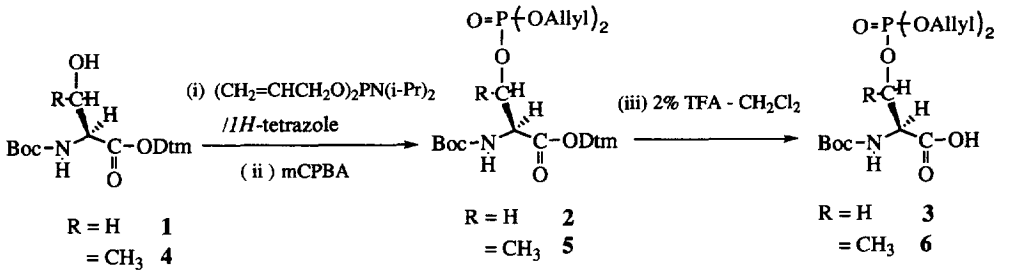
Abstract: The preparation of Boc-Ser(PO_3Allyl_2)-OH and Boc-Thr(PO_3Allyl_2)-OH were described. Phosphorylated RB protein fragments were synthesized using these *O*-phosphorylated amino acid building blocks in the Boc mode of solid phase peptide synthesis.

Phosphorylation of protein is now generally recognized as a major regulatory process in eukaryotic cells[1]. Synthetic peptides phosphorylated at specific site are useful tool for the study of protein phosphorylation. A number of protective groups for phosphate moiety of *O*-phosphoryl serine or threonine have been reported[2]. In a previous paper, we have proposed arylthio group for the protection of phosphate moiety of *O*-phosphoryl serine and threonine building blocks for the synthesis of *O*-phosphoryl peptide by means of the Boc strategy[3]. The diarylthio groups were stable under acidic conditions used in removal of Boc group and could be removed under neutral conditions[4]. However, during the study on the synthesis of *O*-phosphoryl peptides, it was found that *S,S*-bis(*p*-methoxyphenyl)phosphorodithioyl serine was apt to undergo β-elimination because *S,S*-bis(*p*-methoxyphenyl)phosphorodithioyl group is a strong electron-withdrawing group. Recently, Bannwarth *et al.*[5~7] have reported the synthesis of phosphoryl peptides by means of post-phosphorylation approach using allyl group for protection of phosphate moiety. Allyl group is stable under acidic conditions used in removal of Boc group and can be removed under neutral conditions by use of palladium complex[8]. Furthermore, the electron-withdrawing effect of *O*-diallylphosphoryl group is less than that of *S,S*-bis(*p*-methoxyphenyl)phosphorodithioyl group. Thus, *N*-Boc-*O*-diallylphosphoryl serine and *N*-Boc-*O*-diallylphosphoryl threonine seemed to be suitable for the synthesis of *O*-phosphoryl peptide in the Boc mode of solid phase peptide synthesis.

In this paper, we wish to report the synthesis of *N*-Boc-*O*-diallylphosphoryl serine and *N*-Boc-*O*-diallylphosphoryl threonine and demonstrate the synthesis of *O*-phosphorylated peptides using these amino acid building blocks in the Boc mode of solid phase peptide synthesis.



Scheme I

N-Boc-*O*-Diallylphosphoryl-serine was synthesized as follows (Scheme I): *1H*-tetrazole (Tet) (0.20 g, 2.84 mmol) was added to a solution of bis(allyloxy)diisopropropylaminophosphine (0.70 g, 2.84 mmol)[6] and *N*-Boc-serine ditolylmethyl (Dtm) ester (0.87 g, 2.18 mmol)[3] in dry THF (7 ml), and stirred under nitorogen atmosphere at room temperature for 80 min. To the mixture, 70% mCPBA (0.70 g, 2.84 mmol) was added at 0 °C. The solution was diluted with diethyl ether and the ethereal phase was washed with 10% $Na_2S_2O_5$ (25 ml), 5% $NaHCO_3$ (2 × 50 ml), and dried over Na_2SO_4. After silica gel column chromatography (hexane : Et_2O, 1 : 1 v/v), **2**[9] was obtained in 85% yield (1.04 g). Removal of Dtm group from **2** was performed by use of 2% TFA in CH_2Cl_2 at 0 °C for 30 min. After column chromatography (0-5% gradient of methanol in CH_2Cl_2), **3**[10] was obtained in 71% yield (0.34 g). In a similar manner, *N*-Boc-threonine ditolylmethyl ester (**4**) (1.02 g, 2.47 mmol) was phosphorylated to afford **5** in 75% yield (1.07 g). Compound **5** was treated with 2% TFA in CH_2Cl_2 to give **6** in 53% yield (0.42 g).

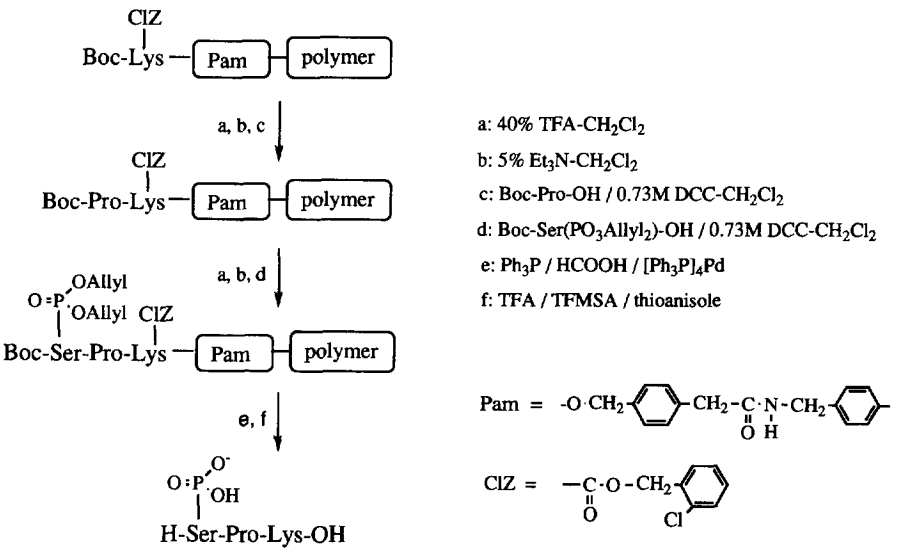


Fig. 1

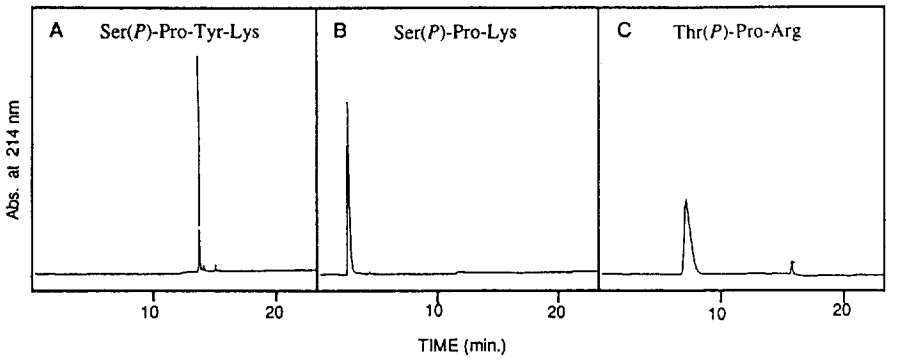


Fig. 2 C18 reversed-phase HPLC profile of the crude product.

To examine the stability of allyl groups, **3** was allowed to stand in TFA in CH_2Cl_2 (1:1, v/v) at room temperature for 2 h, which is the usual condition used in removal of Boc group. Both the ^{31}P-NMR and ^{1}H-NMR spectra of the resulting residue showed that the allyloxy groups were found to be stable under the conditions.

Tumor suppressor retinoblastoma protein (RB protein) is known to be phosphorylated by cdc2-family kinase(s) at multiple serine and threonine residues[11~13]. To examine usefulness of the building block, we have try to synthesize the phosphorylated RB protein fragments. As an example, the synthesis of Ser(*P*)-Pro-Lys is shown schematically in Fig. 1. In this case, lysine-derivatized Pam resin was used. The Boc group of this lysine residue was removed with trifluoroacetic acid. After neutralization, *N*-Boc-proline was coupled with the exposed amino group of lysine residue on the polymer surface. Then, *N*-Boc-*O*-diallylphosphoryl serine was coupled in a similar manner. After the condensation reactions, the peptide was deprotected and removed from the polymer. Two steps of deprotection were performed on the column itself: One ml of 4 M tetrakis-(triphenylphosphine)palladium (Aldrich) in THF, 0.26 g of triphenylphosphine, and 1.88 ml of formic acid in THF (10 ml) were added to the column. After 12 h, the resin was washed three times with 14 ml of CH_2Cl_2, and then treated with 12.5 ml of trifluoromethanesulfonic acid / thioanisole / TFA (2:3:20, v/v/v). After 4 h, the mixture was purified by Sephadex G-15 (1 × 100 cm). Fractions detected by their UV-absorption were collected and lyophilized to obtain the crude product. It was purified by C18 reversed phase HPLC (Fig. 2)[14]. *O*-Phosphoryl serine-containing peptide (**7**) was obtained in 58% yield. In a similar manner, Ser(*P*)-Pro-Tyr-Lys and Thr(*P*)-Pro-Arg were synthesized and obtained in yields of 84% and 25%, respectively. The chromatographic behaviors of Ser(*P*)-Pro-Lys (**7**), Ser(*P*)-Pro-Tyr-Lys (**8**) and Thr(*P*)-Pro-Arg (**9**) were identical with those of authentic samples (Fig. 2)[15] and structures of **7**, **8** and **9** were also supported by its mobility on two-dimentional phosphopeptide mapping.

In conclusion, we have demonstrated the synthesis of the phosphorylated RB protein fragments by employing the *O*-phosphorylated amino acid building blocks such as Boc-Ser(PO_3allyl_2)-OH and Boc-Thr(PO_3allyl_2)-OH. It was found that they were useful for the synthesis of *O*-phosphoryl serine and threonine-containing peptide in the Boc mode of solid phase synthesis.

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