CHEMISTRY LETTERS, pp. 1085-1086, 1979. Published by the Chemical Society of Japan

# A CONVENIENT METHOD OF THE SYNTHESIS OF PHOSPHOPEPTIDE VIA AZIRIDINE RING OPENING REACTION

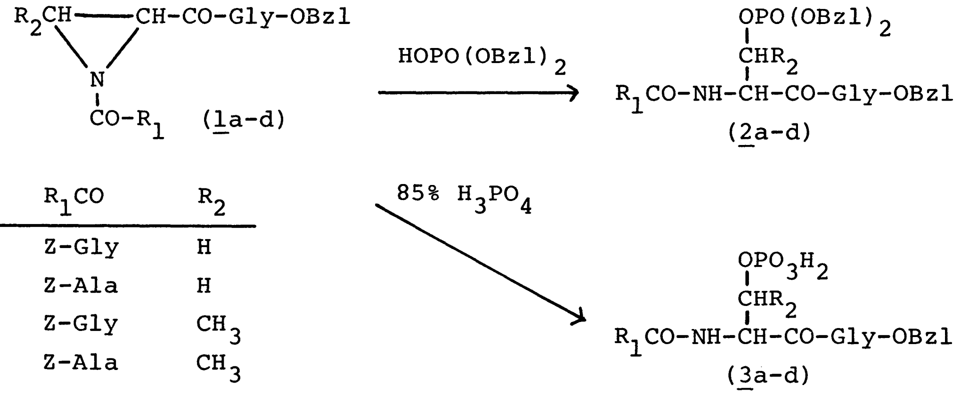
Kenji OKAWA, Masanori YUKI, and Takumi TANAKA
Department of Chemistry, Faculty of Science, Kwansei
Gakuin University, Nishinomiya 662

The reaction of aziridine peptides with dibenzylphosphoric acid and 85% phosphoric acid afforded O-phosphoserine and O-phosphothreonine peptides passing through the ring opening of aziridine in good yields.

Several phosphorylated peptides have been obtained by partial hydrolysis of natural phosphoproteins, which are present in a large variety of animal and plant tissues. By further hydrolysis of these peptides the following simple units have been isolated: O-phosphoserine and O-phosphothreonine. Nowadays, it is well known fact that phosphoric acid directly combine with the hydroxyl group of serine or threonine residue and these phosphate units exist in the parent phosphoproteins. Synthetic approaches in this field of phosphopeptides have been limited only to the simple units. Earlier workers tended to employ the troublesome techniques using alkylphosphorochloridate or allalkylphosphorochloridate.[1,2] These reagents were relatively unstable and gave such units in low yields.

Because of the difficulties of synthesis, we are interested in finding a new method of synthesizing this class of peptides. We reported here the reaction of aziridine peptides including (2S)-2-aziridinecarboxylic acid (Azy) or (2S,3S)-3-methyl-2-aziridinecarboxylic acid (3-Me-Azy) with dibenzylphosphoric acid and 85% phosphoric acid. The aziridine peptides(1) were prepared according to our preceding report.[3]

Phosphorylation of the aziridine peptides(1) to O-dibenzylphosphorylated peptides (2) was easily performed by use of dibenzylphosphoric acid. A typical procedure is as follows. To a solution of 1a (213 mg, 0.5 mmol) in THF (2.5 ml) was added a solution of dibenzylphosphoric acid[4] (278 mg, 1.0 mmol) in THF (2 ml) at -5°C.



|   | $R_1CO$ | $R_2$ |
|---|---|---|
| a | Z-Gly | H |
| b | Z-Ala | H |
| c | Z-Gly | $CH_3$ |
| d | Z-Ala | $CH_3$ |

Table 1. The formation of O-dibenzylphosphopeptides and O-phosphopeptides.

| Compd. | Yield(%) | Mp.(°C) | $[\alpha]_D^{24}$*) | Recrys. Sol. |
|---|---|---|---|---|
| 2a | 72 | oil | -12.7 | |
| 2b | 66 | oil | -12.3 | |
| 2c | 92 | oil | -3.2 | |
| 2d | 64 | oil | -1.4 | |
| 3a | 75 | 148-150 | -8.5 | $CH_3CN$ |
| 3b | 66 | 115-117 | -3.2 | DMF-ether-hexane |
| 3c | 82 | 205(dec.) | +0.8 | Ethyl acetate |
| 3d | 66 | 165(dec.) | +8.5 | DMF-ether |

*) Compounds 2: c 1.0, $CHCl_3$; 3: c 1.0, DMF.
All compounds exhibited satisfying ir and nmr spectra.

After 30 min of stirring, the reaction mixture was set aside at room temperature for 90 min. After removal of the solvent, the residue was dissolved in ethyl acetate and the solution was washed with 1M sodium hydrogencarbonate and water, dried, and concentrated in vacuo. The residue was chromatographed over silica gel by use of ethyl acetate-$CHCl_3$ (1:1 v/v) as eluent; 2a, 254 mg(72%).

On the other hand, O-phosphorylated peptides(3) were directly obtained from the corresponding aziridine peptides(2) by the treatment with 85% phosphoric acid. The following experiment provides a typical procedure for the synthesis of O-phosphorylated peptides. Compound 1a (213 mg, 0.5 mmol) was added to 85% phosphoric acid (8 ml) at -5°C and the reaction mixture was stirred at the same temperature for 2h. Then, saturated NaCl solution (10 ml) was added and the mixture was extracted with ethyl acetate. The extract was washed with water, dried, and concentrated in vacuo. The crystals appeared were collected by filtration, and recrystallized from acetonitrile; 3a, 195 mg(75%).

In conclusion, it was found that this procedure was a convenient method for the synthesis of phosphopeptides and also could be applied to the selective phosphorylation of β-hydroxy amino acid residues in the peptide through aziridine derivatives.

## References and Note

1) G. Fölsch, Acta Chem. Scand., 9, 1039 (1955).
2) G. Riley, J. H. Turnbull, and W. Wilson, J. Chem. Soc., 1957, 1373.
3) K. Okawa, K. Nakajima, T. Tanaka, and Y. Kawana, Chem. Lett., 1975, 591.
Z-Gly-Azy-Gly-OBzl(1a); mp 118-119°C, $[\alpha]_D$-52.5 (c 1.0, MeOH). Z-Ala-Azy-Gly-OBzl (1b); mp 128-128.5°C, $[\alpha]_D$-71.2 (c 1.0, AcOEt). Z-Ala-3-Me-Azy-Gly-OBzl(1d); mp 110-111°C, $[\alpha]_D$-59.7 (c 1.0, $CHCl_3$).
4) F. R. Atherton, H. T. Howard, and A. R. Todd, J. Chem. Soc., 1948, 1106.

(Received June 9, 1979)