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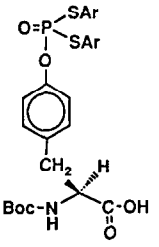
PREPARATION OF *O*-PHOSPHORYL AMINO ACID BUILDING BLOCKS FOR THE SYNTHESIS OF *O*-PHOSPHORYL PEPTIDES

Yoshihito Ueno, Ryuichi Saito and Tsujiaki Hata*

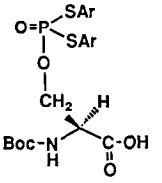
Department of Life Chemistry, Tokyo Institute of Technology,
Nagatsuta, Midoriku, Yokohama 227, Japan

Abstract
Preparation of O-phosphoryl tyrosine, serine, and threonine building blocks for the synthesis of O-phosphoryl peptides is described.

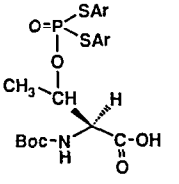
Recently, O-phosphorylation of peptides has been found to act as an important role for cell regulations.[1] In order to synthesize O-phosphoryl peptides, the preparation of suitably protected O-phosphoryl N-tert-butoxycarbonyl (Boc) amino acids, such as **1a**, **1b**, and **1c** is required.



1a



1b

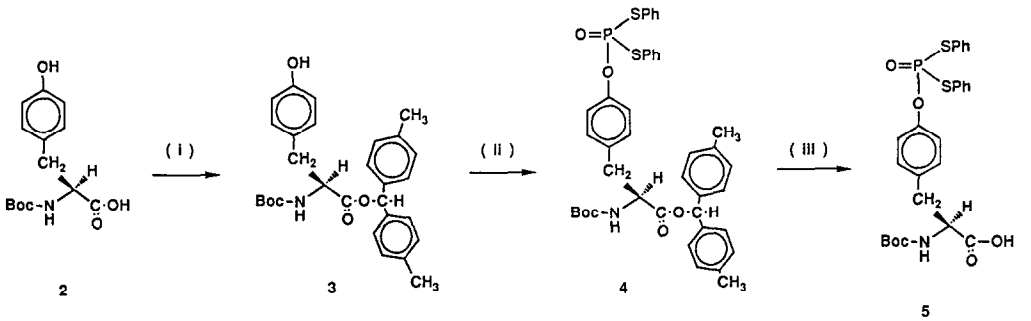


1c

Quite recently, we have reported the synthesis of a dinucleotidyl dipeptide (H-Ala-Tyr(pUpU)-OH)[2] by use of O-(S,S-diphenyl)phosphorodithioyl (PSS) Boc-tyrosine phenacyl ester. The phenacyl group can be removed by treatment with zinc-acetylacetone[2] in pyridine. However, one of two phenylthio groups of PSS residue was released simultaneously under this condition. To avoid the side reaction, a new protecting group for the carboxyl group was required. Ditolylmethyl (Dtm) group was found to be suitable and removed by use of 2% trifluoroacetic acid (TFA) without interfering the Boc and PSS groups.

In this paper, we wish to report the synthesis of carboxyl free O-phosphoryl amino acid building blocks (**1a-c**). In the first place, the synthesis of O-(S,S-diphenyl)phosphorodithioyl[3] Boc-tyrosine (**5**) by using the Dtm group was tried. Boc-tyrosine (**2**) (0.281 g; 1 mmol) was treated with

diazo-4,4'-dimethyldiphenylmethane[5] generated in situ from 4,4'-dimethylbenzophenonehydrazone (0.292 g; 1.3 mmol), 40% peracetic acid (0.25 ml), and iodine (1% w/v solution in CH_2Cl_2; 48μ l) at 0°C to afford the ditolylmethyl ester (**3**) (0.264 g) in 55% yield. It was phosphorylated by using cyclohexylammonium S,S-diphenyl phosphorodithioate[6] (0.318 g; 0.83 mmol) in the presence of isodurenedisulfonyl dichloride (DDS) (0.368 g; 1.1 mmol) and 1H-tetrazole (Tet) (0.078 g; 1.1 mmol) in pyridine (5 ml) to give the fully protected tyrosine (**4**) (0.332 g) in 80% yield. The Dtm group was removed selectively from **4** by use of 2% TFA in CH_2Cl_2 at 0°C for 15 min to afford **5** (0.220 g) in 90% yield.



(i) $(CH_3)_2$-C=N-NH_2, CH_3-C(=O)-OOH, I_2 in CH_2Cl_2, 0°C

(ii) $(PhS)_2$-P(=O)-O⁻, DDS, Tet in pyridine

(iii) 2% TFA - CH_2Cl_2, 0°C

This method seemed to be applied to synthesis of O-phosphoryl Boc-serine and Boc-threonine derivatives. Recently, van Boom[7] reported synthesis of the fully protected N-Boc O-PSS serine where 9,10-anthraquinon-2-yl methyl group was used for protection of the carboxyl group.[8] However, one of two phenylthio groups was lost during the removal of the anthraquinon-2-yl methyl group. On the contrary, the Dtm group can be removed under acidic conditions (2% TFA). Therefore, we would be able to prevent the loss of the phenylthio group by an attack of the carboxyl group. In order to test the stability of the arylthio groups, we have prepared several kinds of S,S-diaryl phosphorodithioate derivatives of Boc-serine and Boc-threonine.

O-(S,S-Diaryl)phosphorodithioyl Boc-serine and Boc-threonine derivatives [R=H (**8a-d**), R=CH_3 (**8e-h**)] were prepared by a similar procedure as described in the case of Boc-tyrosine derivatives (**5**). The yields and ^{31}P-NMR data for **8a-h** are summarized in Table 1.

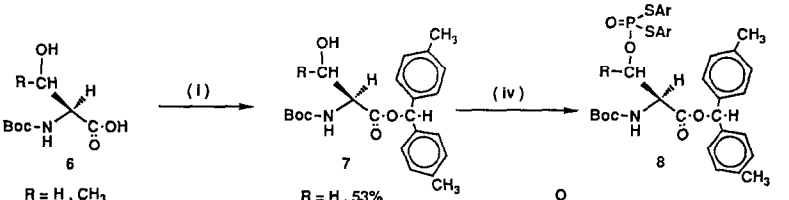
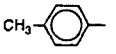
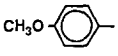


Table 1

Ar	R = H (Ser)			R = CH_3 (Thr)		
		yield (%)	^{31}P NMR (ppm)*		yield (%)	^{31}P NMR (ppm)*
	8a	76	50.33	8e	88	47.52
	8b	73	51.11	8f	76	48.30
	8c	75	48.83	8g	72	46.60
	8d	81	50.67	8h	82	48.73

*solvent : $CDCl_3$

Removal of the Dtm group from **8** was performed by use of 2% TFA in CH_2Cl_2 at 0°C for 15 min. After column chromatography (0-2.5% gradient of methanol in CH_2Cl_2), **9** was obtained. The yields and ^{31}P-NMR data for **9a-h** are summarized in Table 2. In the case of **9a-c**, yields were relatively low

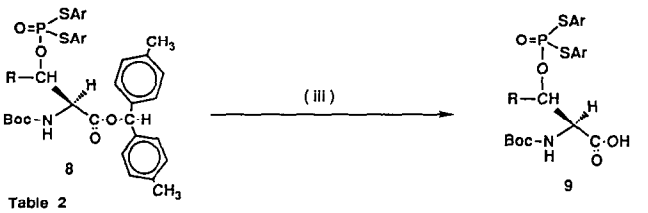
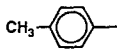
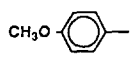


Table 2

Ar	R = H (Ser)			R = CH_3 (Thr)		
		yield (%)	^{31}P NMR (ppm)*		yield (%)	^{31}P NMR (ppm)*
	9a	59	51.79	9e	89	49.12
	9b	64	53.19	9f	83	49.36
	9c	69	50.62	9g	85	47.33
	9d	82	52.75	9h	81	50.33

*solvent : $CDCl_3$

and it may be due to the instability of **9a-c**. When **9c** was allowed to stand in pyridine-$CHCl_3$ (2:1, v/v) at room temperature for 1 h, three peaks appeared in the ^{31}P-NMR spectrum (Fig. 1). These peaks at 14.05, 27.56 and 45.73 ppm were assigned as unwanted O-(S-o-tolyl)phosphorothioyl Boc-serine, a mixed anhydride between S-o-tolyl phosphorothioic acid and the carboxylic acid of Boc-serine, and S,S-di-o-tolyl phosphorodithioate, respectively. Similar phenomena were observed in the case of **9a**, **9b** and **9e-g**. However, it is noted that **9d** and **9h** having O-bis(S,S-p-methoxyphenyl)-phosphorodithioyl group, were stable enough under these conditions.

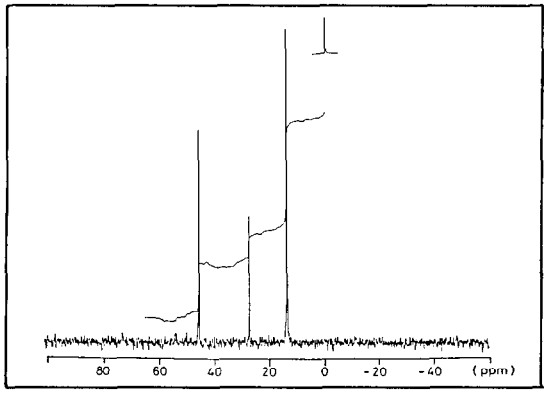


Fig. 1 ^{31}P-NMR spectrum of 9C after treatment with pyridine-$CHCl_3$ (2:1 v/v) for 1 hour.

In conclusion, O-bis(S,S-p-methoxyphenyl)phosphorodithioyl derivatives of Boc-serine and Boc-threonine, **9d** and **9h** are useful building blocks for the synthesis of the phosphoryl peptides. In the case of tyrosine, the PSS derivative (**5**) is possible to use as the building block.

References and Notes

1) P. Cohen, Nature, 296, 613 (1982).
2) H. Hotoda, Y. Ueno, M. Sekine, and T. Hata, Tetrahedron Lett., 30, 2117 (1989).
3) The phenylthio groups can be removed smoothly by treatment with silver nitrate under neutral conditions.[4]
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