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Improved Protective Groups for Phosphate of O-Phosphoserine Useful for the Solid-Phase Peptide Synthesis[1]

Masamitsu Tsukamoto, Rika Kato, Koichi Ishiguro, Tsuneko Uchida, and Kazuki Sato*

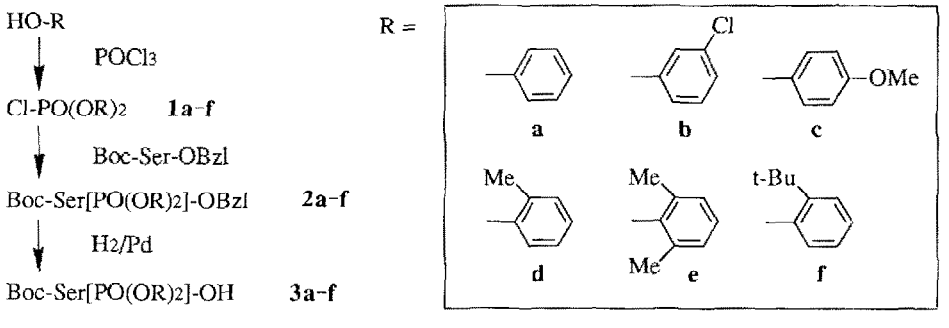
Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan

Key words: Phosphopeptide; Solid phase synthesis; Protective group; HF treatment; Substituted phenyl

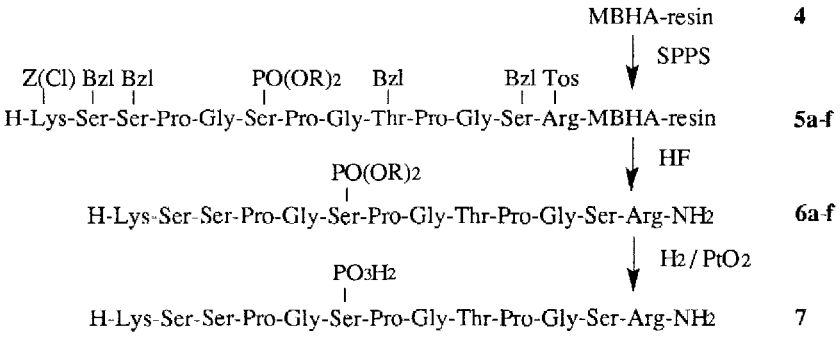
Abstract: *2-Methylphenyl and 2,6-dimethylphenyl groups on phosphate moiety of O-phosphoserine were more stable in HF treatment than phenyl group and could be effectively removed by hydrogenolysis. They were shown to be protective groups for phosphoserine practically applicable in the Boc/Bzl mode of solid-phase peptide synthesis.*

Synthetic peptides phosphorylated at specific site are useful for the study of protein phosphorylation[2], and the demand for various phosphorylated peptides has been increasing. Many protective groups for phosphate moiety of phosphorylated serine or threonine have been reported[3]. Among them, phenyl group (**a**) is widely used in the Boc/Bzl mode of solid-phase peptide synthesis (SPPS) and reported to be stable to HF cleavage from polymer supports[3e]. We observed considerable dephosphorylation during HF treatment, however. In search of more stable protective groups, several substituted phenyl groups were examined utilizing phosphoserine (Scheme 1).

Diaryl phosphochloridates **1b–f** synthesized by the Rosenmund-Vogt method[4] and purified by distillation of the reaction mixture under reduced pressure[5] were reacted with Boc-Ser-OBzl to provide Boc-Ser[PO(OR)2]-OBzl **2b–f**[6] as reported for **2a**[3e]. Addition of dimethylaminopyridine was necessary to accelerate the reaction in the synthesis of **2e** and **2f**. The benzyl group in **2a–f** was removed by hydrogenolysis on Pd/C to give Boc-Ser[PO(OR)2]-OH **3a**[3e], **3b–f**[7] in quantitative yield.



Scheme 1. Synthesis of Protected Phosphoserine Derivatives (**3a–f**).



Scheme 2. Solid Phase Synthesis of [Lys[139], Ser(PO_3H_2)[144]]tau(139-151)NH_2 (**7**).

To compare the usefulness of these protective groups, we synthesized phosphorylated tau protein fragment [Lys[139], Ser(PO_3H_2)[144]]tau(139-151)NH_2 (**7**)[8] as shown in Scheme 2 and the results are summarized in Table 1. Protected peptide resins **5a–f** were prepared from MBHA-resin (**4**) in almost quantitative yields by the Boc/Bzl mode of SPPS and treated with HF at 0 °C for 30 min to liberate peptides with protected phosphate group (**6a–f**)[9]. When peptide resin **5a** was treated with HF, the crude extract showed two major peaks on analytical HPLC (Fig. 1a)[10]. Peaks A and B correspond to **6a** and its dephosphorylated form, respectively. The result indicates that dephosphorylation takes place in HF at 0 °C. 3-Chlorophenyl (**b**) and 4-methoxyphenyl (**c**) groups did not retard the dephosphorylation, but, with 2-methylphenyl group (**d**), the amount of dephosphorylated peptide decreased. 2,6-Dimethylphenyl (**e**) was more effective in preventing the dephosphorylation and in the case of 2-*tert*-butylphenyl (**f**) group, no dephosphorylation was observed.

In addition to HF treatment, **6a** slowly decomposed in aqueous solution during HPLC purification, resulting in low isolation yield. We could not isolate **6b**, because it was quite unstable. On the other hand, **6d–f** were more stable than **6a** and resulted in higher isolation yields. These results suggest that steric factor is important for the stability of these protective groups.

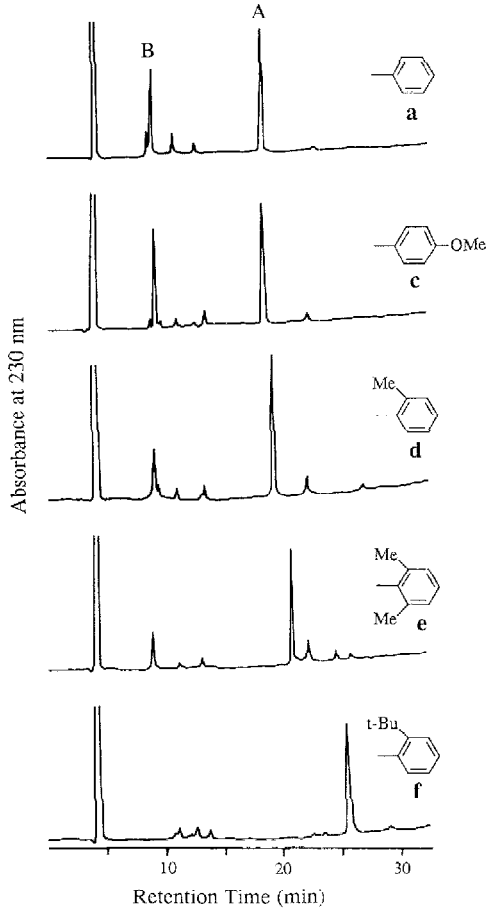


Figure 1. HPLC of crude extracts after HF treatment of **5a**, **5c–f**.

Table. 1. Yields[a] of Protected (**6a–f**) and Deprotected (**7**) Peptides by the Use of Boc-Ser[PO(OR)2]-OH

R	Protected peptides (**6a–f**)		Deprotected peptide (**7**)
	Ratio[b] (P : DP)	Yield from **4** (%)	Yield (%)
Phenyl (**a**)	(60 : 40)	23	68
3-Chlorophenyl (**b**)	(33 : 67)	NI[c]	
4-Methoxyphenyl (**c**)	(57 : 43)	18	
2-Methylphenyl (**d**)	(72 : 28)	46	65
2, 6-Dimethylphenyl (**e**)	(80 : 20)	52	42
2-*tert*-Butylphenyl (**f**)	(100 : 0)	55	29

[a] Isolation yields after preparative HPLC.

[b] P (Phosphorylated peptide) and DP (Dephosphorylated peptide) in crude extracts after HF treatment.

[c] Not isolated because of low stability.

Phenyl group of **6a** was removed by hydrogenolysis under H_2 (5-6 atm) with PtO_2 catalyst to give **7**[11]. The yield starting from MBHA-resin was 16%. Hydrogenolysis of **6d** proceeded as smoothly as in **6a** within 12 hrs and the yield from MBHA-resin was 30%. 2,6-Dimethylphenyl group (**e**) was a little resistant to hydrogenolysis and it took 48 hrs for complete deprotection. In the case of 2-*tert*-butylphenyl group (**f**), the reductive cleavage was slow and considerable amount of side products gave lower step yield[12]. The stability of protective groups in HF treatment appears to relate to the difficulty of their final deprotection by hydrogenolysis.

In summary, 2-alkylphenyl groups were more stable than ordinary phenyl group as protective groups on phosphate moiety of O-phosphoserin. 2-Methylphenyl and 2,6-dimethylphenyl groups are recommendable. These new protective groups should be useful in the Boc/Bzl mode of solid phase synthesis of O-phosphopeptide, especially, of the peptide with several phosphorylated residues.

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REFERENCES AND NOTES

1. The abbreviations used in this paper are those recommended by IUPAC-IUB: *Eur. J. Biochem.* **1984**, *138*, 9-37. Additional abbreviations: Bzl, benzyl; Boc, tert-butoxycarbonyl; Tos, p-toluensulfonyl; Z(Cl), 2-chlorobenzyloxycarbonyl; MBHA, *p*-methylbenzhydrylamine.
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5. **1b**: yield 61%; bp 200-210 °C / 0.5 mmHg. **1c**: yield 50%; bp 195-200 °C / 0.5 mmHg. **1d**: yield 67%; bp 145-150 °C / 0.03 mmHg. **1e**: yield 53%; bp 173 °C / 0.2 mmHg. **1f**: yield 43%; bp 185-190 °C / 0.2 mmHg.
6. **2b**: yield 46%; mp 82-84 °C **2c**: yield 57%; oily substance **2d**: yield 69%; mp 85-87 °C **2e**: yield 76%; oily substance **2f**: yield 68%; oily substance. MS and ^{1}H-NMR ($CDCl_3$) spectra of these compounds showed reasonable results. Elemental analysis of **2b** and **2d** also agreed with calculated values.
7. Structure of **3b–f** were confirmed by MS and ^{1}H-NMR ($CDCl_3$) spectra.
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9. **6a**: FAB-MS MH^+ 1445, Calcd for $C_{61}H_{93}O_{21}N_{18}P$ Mw 1445. Amino acid analysis, Thr, 0.93 (1); Ser, 3.10 (4); Pro, 2.70 (3); Gly, 3.00 (3); Lys, 0.98 (1); Arg, 1.00 (1). **6c**: FAB-MS MH^+ 1506, Calcd for $C_{63}H_{97}O_{23}N_{18}P$ Mw 1505. **6d**: FAB-MS MH^+ 1474, Calcd for $C_{69}H_{97}O_{21}N_{18}P$ Mw 1473. **6e**: FAB-MS MH^+ 1502, Calcd for $C_{65}H_{101}O_{21}N_{18}P$ Mw 1501. **6f**: FAB-MS MH^+ 1558, Calcd for $C_{69}H_{109}O_{21}N_{18}P$ Mw 1557.
10. Linear gradient from 5% to 65% CH_3CN in 0.1% TFA in 30 min. Flow rate 1ml / min. ODS column 4.6 x 250 mm. Large peak at 4 min is solvent (AcOH) of samples.
11. A mixture of **6a** (60 mg) and PtO_2 (60 mg) in AcOH (1 ml) was stirred under H_2 (5-6 atm) for 12 hrs at room temperature. The catalyst was removed by filtration, and the filtrate was lyophilized and purified by preparative HPLC to give phosphopeptide **7** (37 mg); FAB-MS MH^+ 1294, Calcd for $C_{49}H_{85}O_{21}N_{18}P$ Mw 1293. Amino acid analysis, Thr 1.10 (1); Ser 3.30 (4); Pro 3.40 (3); Gly 2.9 (3); Lys 1.02 (1); Arg 1.06(1).
12. Reduction of **6f** proceeded only 50% after 72 hr reaction with large amount of additional PtO_2.

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