

## A New Approach to Phosphoserine and Phosphothreonine Synthons Suitable for the Stepwise Synthesis of Phosphopeptides.

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**Abstract :** Several phosphoserine and phosphothreonine synthons suitable for the stepwise synthesis of phosphopeptides have been prepared. Treatment of methylthiomethyl (MTM) esters of either benzyloxycarbonyl (Z), *tert*-butyloxycarbonyl (Boc) and allyloxycarbonyl (Alloc) serine and / or threonine with phosphochloridate in pyridine, followed by MgBr<sub>2</sub> cleavage of MTM in diethylether, afforded the title compounds in excellent yields.

Reversible protein phosphorylation is widely recognized as an important mechanism for the regulation of many cellular processes. Specific serine, threonine or tyrosine residues in protein substrates are phosphorylated/dephosphorylated by kinases and phosphatases respectively. Many biological important proteins such as enzymes, growth factor receptors, cytoskeletal and contractil proteins, proteins acting in the cellular cycle, oncogenic proteins are known to exist as phosphoproteins <sup>1-9</sup>. In addition, major proteins of bone, teeth, eggs and milk are also highly phosphorylated <sup>1</sup>. Synthetic phosphopeptides related to biological phosphoproteins are increasingly used as models to study various aspects of proteins structure and functions.

Therefore, it is of considerable interest to develop efficient synthetic routes to phosphopeptides <sup>2</sup>.

Three general approaches can be envisaged :

- 1) Enzymatic phosphorylation of synthetic peptides (hemi-synthetic strategy). This method is attractive but limited to very specific peptide sequences <sup>2</sup>.
- 2) Chemical phosphorylation of preformed synthetic peptides ("global strategy") <sup>2</sup>.
- 3) Incorporation of phosphoaminoacid derivatives in the growing peptides ("sequential or stepwise strategy") <sup>2</sup>.

The most general stepwise strategy requires the preparation of adequately protected phosphoaminoacid derivatives bearing a free carboxyl group. This route is suitable for both liquid and solid-phase peptide methodologies. The poor stability of phosphoserine and phosphothreonine which are readily converted into the corresponding  $\alpha,\beta$ -dehydro derivatives<sup>10</sup> makes the purification very tedious and limits the choice of the protecting groups. The most common approach consists in the phosphorylation of N- and C-protected serine and/ or threonine residues followed by mild selective deprotection of carboxyl terminus <sup>2</sup>. Recently, a new strategy was proposed, the so-called "both ends deprotection" <sup>11</sup> which involves the phosphorylation of N- and C-protected serine followed by the deprotection of both amino and carboxyl functionalities and finally reprotection of the amino terminus.



N-protected serine and/or threonine **1** were esterified by treatment with a mixture of DMSO, *t*-butylbromide, NaHCO<sub>3</sub><sup>14</sup> to afford after flash silica-gel column chromatography pure MTM esters **2a-2f**. The latter were phosphorylated by the phosphochloridate methodology using two commercially available reagents : diphenyl phosphochloridate<sup>15</sup> and bis-(2,2,2-trichloroethyl) phosphochloridate<sup>16</sup>. These phosphate protecting groups are compatible with the usual amino-terminus protections: benzyloxycarbonyl (Z), *t*-butyloxycarbonyl (Boc) and allyloxycarbonyl (Alloc).

The reaction was carried out in pyridine at -20°C for serine derivatives and at room temperature for threonine analogs. Fully protected phosphoaminoacids **3a-3f** were obtained in good yield and used without purification. The structure of compounds **3a-3f** was ascertained by <sup>31</sup>P-, <sup>13</sup>C- and <sup>1</sup>H-NMR spectroscopy. It should be noted that no α,β-dehydro derivatives were detected (i.e., <sup>1</sup>H-NMR monitoring). MTM protecting group was smoothly removed by treatment with magnesium bromide in ether<sup>17</sup> to afford the expected carboxyl-free phosphoaminoacids **4a-4l** with yield ranging from 75 to 95%.

Serine derivatives were generally obtained as crystalline compounds (table I). In contrast, threonine analogs were recovered as oily materials, isolated and purified as the dicyclohexylammonium (DCHA) salts. All compounds gave satisfactory elemental analysis and were fully characterized by NMR spectroscopy (<sup>1</sup>H, <sup>13</sup>C, <sup>31</sup>P).

**Table 1** Partial physical constants for phosphoserine derivatives (**4a-4f**) R<sub>2</sub> = H (\* DCHA salt)

Compounds	<b>4a</b> <sup>i</sup>	<b>4b</b>	<b>4c</b>	<b>4d</b> <sup>ii</sup>	<b>4e</b> <sup>iii</sup>	<b>4f</b>
[α] <sub>D</sub> <sup>20</sup> CHCl <sub>3</sub> , C=1	+ 30	+ 18 *	+ 22 *	+ 18	+ 27	+ 23
mp °C	84	118*	97*	153	143	88
<sup>31</sup> P NMR <sup>a</sup> ppm (δ)	- 11.9	- 11.47	- 11.46	- 4.3	- 4.43	- 4.51

<sup>a</sup> CDCl<sub>3</sub> was used as the solvent.

<sup>i</sup> lit. [α]<sub>D</sub><sup>20</sup> : + 40 (c = 1, CHCl<sub>3</sub>) mp : 62-64 °C<sup>2,18</sup>

<sup>ii</sup> lit. [α]<sub>D</sub><sup>28</sup> : + 23.8 (c = 0.58, CHCl<sub>3</sub>) mp : 145 °C<sup>11</sup>

<sup>iii</sup> lit. [α]<sub>D</sub><sup>28</sup> : + 26.6 (c = 0.30, CHCl<sub>3</sub>) mp : 141 °C<sup>11</sup>

**Table 2** Partial physical constants for phosphothreonine derivatives (**4g-4l**) R<sub>2</sub> = CH<sub>3</sub> (DCHA salt)

Compounds	<b>4g</b>	<b>4h</b>	<b>4i</b>	<b>4j</b>	<b>4k</b>	<b>4l</b>
[α] <sub>D</sub> <sup>20</sup> CHCl <sub>3</sub>	+ 15 C = 1	+ 6 C = 1	+ 8 C = 1	+ 0.1 C = 0.7	+ 13 C = 0.7	+ 0.2 C = 0.9
mp °C	134.5	93	108	119	118	105
<sup>31</sup> P NMR <sup>a</sup> ppm (δ)	- 12.58	- 12.47	- 12.53	- 4.98	- 5.12	- 5.06

<sup>a</sup> CDCl<sub>3</sub> was used as the solvent.

In conclusion, the method described herein allowed us to prepare a series of phosphoserine and phosphothreonine derivatives in good yield. It should be a very useful complement to the few existing chemical methods. In our hand it proved very convenient for the preparation of phosphoserine and phosphothreonine building blocks.

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#### References and notes

- 1 Weller, M. *Protein Phosphorylation*, Pion Ltd.: London. 1979.
- 2 Kemp, B. E. Ed. CRC Press, *Peptides and protein phosphorylation*, 1990, 289 - 314.
- 3 Cohen, P. *Nature*, 1982, 296, 613-620.
- 4 Ingebritten, T. S.; Cohen, P. *Science*, 1983, 221, 331-338.
- 5 Cohen, P. *Eur. J. Biochem.*, 1985, 151, 439-448.
- 6 Shenolikar, S. *J. Cyclic Nucl. Pro. Phys. Res.*, 1987, 11, 531-541.
- 7 Edelman, A. M.; Bumenthal, D. K.; Krebs, E. G. *Ann. Res. Biochem.*, 1987, 56, 567-613.
- 8 Haganir, R. L.; Greengard, P. *TIPS*, 1987, 8, 472-477.
- 9 Shenolikar, S. *FASEB J.*, 1988, 2, 2753-2764.
- 10 Paquet, A. *Tetrahedron Lett.*, 1990, 31, 5269-5272.
- 11 Paquet, A. *Int. J. Peptide. Protein Res.*, 1992, 39, 82-86.
- 12 Ross, C.E.; Barnabé, P.; Hiemstra, H.; Speckamp, W.H. *Tetrahedron Lett.*, 1991, 32, 6633-6636.
- 13 Lacombe, J.M.; Andriamanampisoa, F.; Pavia A.A. *Int. J. Peptide. Protein Res.*, 1990, 36, 275-280 :  
the alloc protecting group can also be removed by the hydrostannolytic method.
- 14 Dossena, A.; Palla, G.; Marchelli, R.; Lodi, T. *Int. J. Peptide Protein Res.*, 1984, 23, 198-202.
- 15 The phenyl group can be removed by catalytic hydrogenation (PtO<sub>2</sub>) in trifluoroacetic acid. The Z group can be removed by hydrogenation (Pd/C) without affecting the phenyl group of phosphate.(Ref 2)
- 16 Trichloroethyl group can be removed by Zn / Acetic Acid ( Paquet A.; Johns, M. *Int. J. Peptide Protein Res.*, 1990, 36, 97-103) or by hydrogenolysis in aqueous ethanol (ref 11).
- 17 Kim, S.; Park, Y.H.; Kee, I.S. *Tetrahedron Lett*, 1991, 32 , 3099-3102.
- 18 Perich, J.W.; Alewood, P.F.; Johns, R.B. *Synthesis*, 1986, 572-573.

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