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FMOC Solid Phase Synthesis of Serine Phosphopeptides via Selective Protection of Serine and On Resin Phosphorylation

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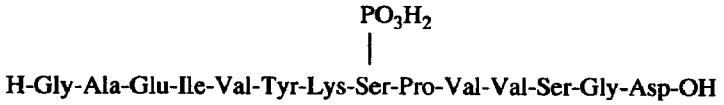
Summary: An Fmoc based solid phase method for the synthesis of phosphoserine peptides has been developed which is amenable to standard protocols on automated synthesizers. The serine residue to be phosphorylated is protected with a t-butyldimethylsilyl group which is selectively removed from the resin bound protected peptide. On resin phosphorylation and final deprotection/cleavage are performed in standard fashion to yield serine phosphopeptides of significant length and complexity.

In the last few years significant effort has been directed toward the synthesis of phosphopeptides and isosteres thereof due to the important role of protein phosphorylation in biology.[1] Clearly, solid phase peptide synthesis (SPPS) methodology is to be preferred for phosphopeptides which are longer than just a few residues and for purposes of automation. The SPPS of phosphoserine peptides using protected serine phosphate ester amino acid building blocks has met with difficulty.[2] Fmoc-synthesis[3] is precluded due to facile beta elimination to give dehydroalanine. Boc-SPPS[4] of phosphopeptides is problematic due to competing phosphate cleavage during the cleavage of the peptide from Merrifield resin which requires very strong acid.[5] By far the most practical method for preparing phosphoserine peptides has been Fmoc-SPPS followed by post synthetic phosphorylation of the free hydroxyl group of serine which is left unprotected during the synthesis.[6] In order to avoid competing O-acylation of serine during chain elongation pentafluorophenyl (Pfp) or 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (Dhbt) esters have been used. Nevertheless, the use of unprotected serine as reported could be problematic for longer sequences having C-terminal serine and therefore many subsequent coupling steps. Furthermore, we desired a method which would be more flexible with regard to the coupling reaction and in particular compatible with carbodiimide mediated peptide bond formation.[7] A simple strategy was conceived to address the above concerns. It seemed likely that with the t-butyldimethylsilyl (TBDMS) group selective protection of a given serine residue relative to all other protected amino acid functionalities for Fmoc SPPS could be achieved.[8] This protecting group should be stable to the conditions of Fmoc SPPS and be compatible with most coupling methods. After completion of the synthesis with the peptide still on the resin we anticipated that the TBDMS group might be selectively cleaved with fluoride unmasking the serine hydroxyl to be phosphorylated. At this point phosphorylation of the serine hydroxyl could be achieved by any of the reported methods with the phosphoramidate method being apparently preferred.[9] Herein, we report the successful application of this strategy to tau phosphopeptide sequence, **1**.[15]

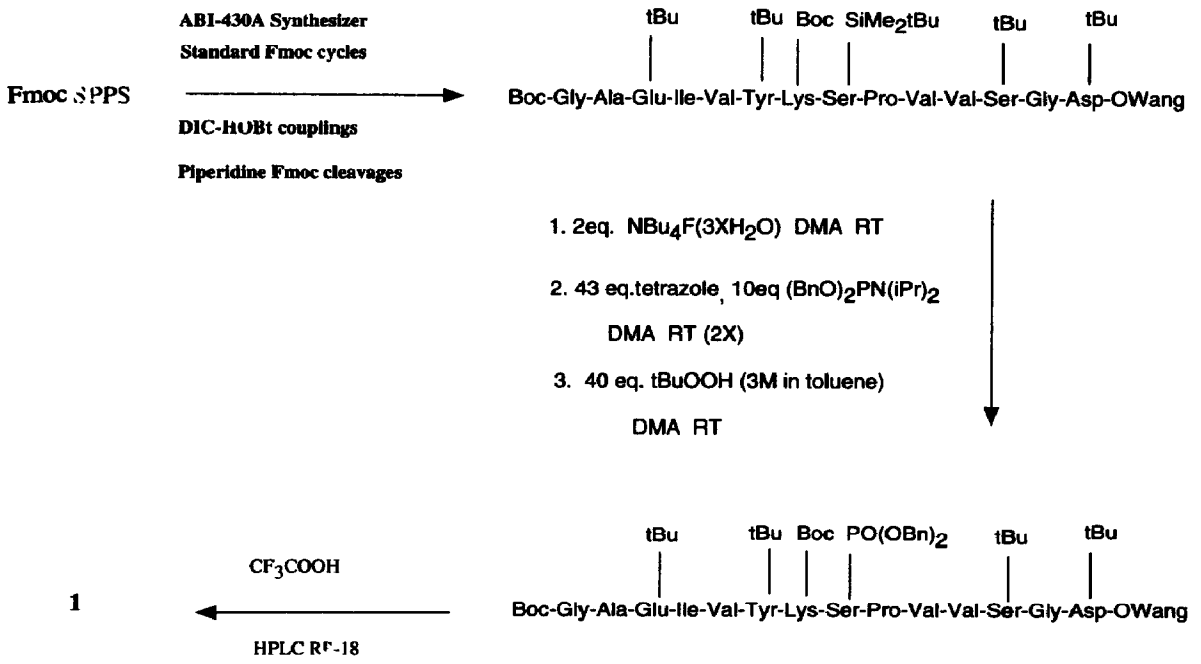
We wished to prepare the human tau sequence 389-402 with phosphoserine at position 396 (Figure 1). The normal peptide sequence was prepared on an Applied Biosystems 430A peptide synthesizer using the standard SPPS-Fmoc cycles provided by ABI. Thus, couplings were mediated



1 Human tau (389-402) with Ser(P) at position 396

Figure 1

with hydroxybenzotriazole-diisopropylcarbodiimide-DMF and Fmoc cleavages with piperidine-DMF. Asp-Wang resin[10] (0.50mmol, 0.54mmol/g loading) was employed and t-butyl protection was used for the functional amino acids (Tyr(tBu), Glu(tBu), Asp(tBu), Lys(Boc), Ser(tBu)) with the exception of Ser396 which was protected as its t-butyldimethylsilyl ether.[11] Double couplings were performed for



Scheme 1

amino acids 391-399, and the N-terminal glycine residue was incorporated with Boc protection for the amino group (Scheme 1).

After the peptide chain assembly was complete the 0.50mmol of resin was treated with 2.0eq tetrabutylammonium fluoride trihydrate in dimethylacetamide (DMA) at room temperature for one hour. The resin was washed four times with DMA twice with toluene and then treated with tetrazole (43eq) and dibenzyl-N,N-diisopropyl-phosphoramidate[6a,12] (10eq) in DMA at room temperature for two

hours twice in succession. Oxidation was then performed with 40eq. t-butylhydroperoxide (3M in toluene) in DMA at room temperature for one hour. The peptide was then cleaved off the resin in the

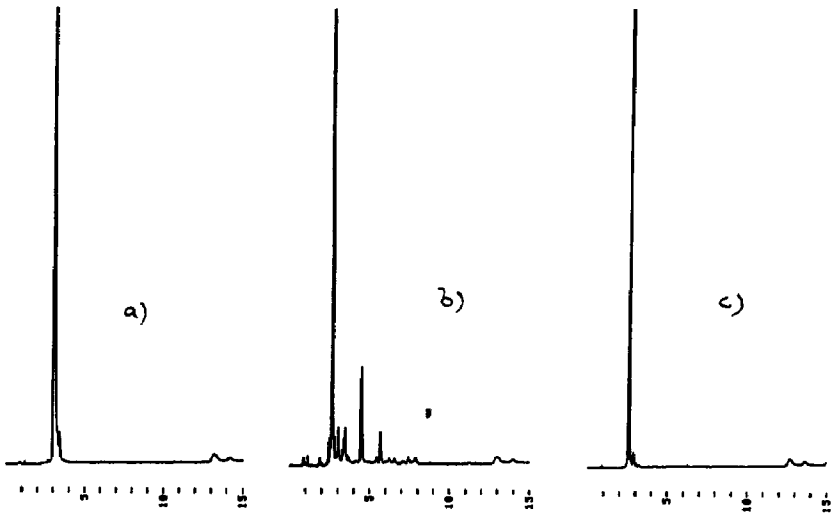


Figure 2

a) crude unphosphorylated peptide, b) crude phosphorylated peptide, c) purified phosphorylated peptide

HPLC-Chromatogramms:
Semipreparative HPLC: Hyperchrome 2025, Nucleosil C 8 column (10 my, 20x250 mm); solvents: (A) 0.1% H3PO4 in water, (B) 0.1% H3PO4 in MeCN; flow rate 7.5 ml/min; gradient: within 50 min from 5% B to 75% B; detection 205 nm.
Analytical HPLC profile: LiChroCART, LiChrospher 100 RP-18 column (5 my, 4x125 mm); solvents: (A) H2O/MeCN/H3PO4 95:5:0.1, (B) 0.1% H3PO4 in MeCN; flow rate 1.5 ml/min; gradient: within 20 min from 0% B to 50% B; detection 205 nm.

standard manner[3] with trifluoroacetic acid: water (95:5). This gave 643mg of crude phosphopeptide (HPLC Figure 2). Semipreparative HPLC purification of a 90mg aliquot of the crude phosphopeptide followed by ion exchange chromatography gave 33mg (32%) of lyophilized phosphopeptide **1**.[13] HPLC analysis indicated >95% purity (Figure 2) for **1** which gave satisfactory FAB-MS (MH^+= 1501) and amino acid analysis.[14]

In conclusion, a method for the synthesis of serine phosphopeptides has been developed which is compatible with standard carbodiimide based Fmoc-SPPS protocolls on automated synthesizers. Regarding the generality and limitations of the method, further studies are required.

REFERENCES

1. de Bont, D. B. A.; Moree, W. J.; van Boom, J. H. and Liskamp, R. M. J. *J. Org. Chem.* **1993**, *58*, 1309. and references therein.
2. Perich, J. W. "Modern Methods of O-Phosphoserine and O-Phosphotyrosine Containing Peptide Synthesis" in *Peptides and Protein Phosphorylation,* CRC Press, 1990, ed. Kemp, Bruce E.
3. Atherton, E. and Sheppard, R. C. *Solid Phase Peptide Synthesis*; Richwood, D., Hamas, B. D. Eds.: The Practical Approach Series; Information Press Ltd. Oxford, 1989.
4. Stewart, J. M. and Young, J. D. *Solid Phase Peptide Synthesis*; Pierce Chemical Co. (Rockford, Illinois),1984.
5. Tsukamoto, M.; Kato, R.; Ishiguro, K.; Uchida, T. and Sato, K. *Tetrahedron Lett.* **1991**, *32*, 7083.
6. a. Andrews, D. M.; Kitchin, J.; and Seale, P. W. *Int. J. Peptide Protein Res.* **1991**, *38*, 469. b. de Bont, H. B. A.; van Boom, J. H.; and Liskamp, R. M. J.; *Tetrahedron Lett.* **1990**, *31*, 2497. c. Staerker, G.; Jakkobsen, M. H.; Olsen, C. E.; and Holm, A. *Tetrahedron Lett.* **1991**, *32*, 5389.
7. Carbodiimide mediated couplings are standard on the ABI-430A automated peptide synthesizer and can also be used on most commercial peptide synthesizers.
8. In the course of our studies the application of t-butyldimethylsilyl ethers of serine, threonine and tyrosine in Fmoc-SPPS of standard peptides was described and the implications for phosphopeptide and glycopeptide synthesis noted: Fischer, P. M. *Tetrahedron Lett.* **1992**, *33*, 7605.
9. The direct phosphorylation of the free serine hydroxyl group of peptides with dibenzylphosphochloridate has been described: Otvos, L.; Elekes, I. and Lee, V.M-Y. *Int. J. Protein Res.* **1989**, *34*, 129. However, the use of phosphoramidate chemistry, phosphitylation followed by oxidation, appears to be a more reliable method.[6a]
10. Wang, S. *J. Am. Chem. Soc.* **1973**, *95*, 1328. Wang-Asp was purchased from Novabiochem.
11. L-Fmoc-Ser(tBuSiMe$_2$)-OH was purchased from Bachem, Switzerland.
12. We have found that this material can be distilled (bulb to bulb, b.p. 185-195°C, 0.1mm) avoiding the rather lenghty chromatographic purification of this hydrolytically unstable material.
13. The 90mg were purified in two 45mg portions under the conditions described in Figure 2. For the ion exchange chromatography (desalting) Biorad AG 4X-4, 100-200 mesh was used with 2NAcOH eluent.
14. From the amino acid analysis the peptide content of purified **1** was determined to be 73%
15. This compound has been mentioned in Lee, V.M-Y.; Balin, B.J.; Otvos, L. and Trojanowski, J.Q. *Science* **1991**, *251*, 675 but no experimental details concerning the synthesis were given.

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