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[A0030]

Synthesis of Protected Thiophospho Amino Acids for Solid Phase Peptide Synthesis

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Abstract: The synthesis of protected thiophospho amino acids, either as thiophosphotriesters or -diesters, conceived as building blocks for solid-phase synthesis of thiophosphorylated peptides is described. The thiophosphotriesters **4**-**9**, namely *O,O*-diprotected, -thiophosphorylated *N*-Alloc, *N*-Fmoc and *N*-Boc protected serine, threonine and tyrosine, were prepared in a one-pot procedure from the corresponding *N*-protected amino acids. From *O,O*-dibenzyl *N*-Fmoc serine thiophosphate **7**, either *S*-benzyl (**10**) or *O*-benzyl (**11**) thiophosphodiesters can be obtained.

Keywords: Thiophospho Amino acids.

The reversible phosphorylation of proteins and peptides is a crucial reaction in biological regulation. In compounds which are recognised as substrates of biological phosphoryl transfer reactions, replacement of a phosphoryl oxygen by a sulfur may result in selective inhibitors of these proceses.[1] In this context, we have been interested in the preparation of thiophosphate analogues of peptides containing phosphoserine or phosphothreonine, employing *O*- or *S*-phospho-protecting groups such as benzyl or allyl.

Until now, the most common method for preparing *O*-thiophosphorylated peptides at serine, threonine or tyrosine residues has been the incubation of the peptide or protein with a suitable kinase and g-*S*-ATP.[1] In spite of their potential interest as biological tools, comparatively few reports attempting non-enzymatic synthesis of peptide thiophosphates have appeared. Two approaches have been followed, namely the on-resin phosphitylation-oxidation of a suitably protected peptide and the incorporation of a protected thiophosphorylated building block by traditional Boc synthesis methods into a peptide sequence.[2] The advantage of on-resin, post-assembly, "global" thiophosphorylation is its convenience for simple sequences, but may pose difficulties in the synthesis of multiply (thio)phosphorylated substrates, or those in which a combination of specifically phosphorylated and thiophosphorylated sites is desired.[3] For this purposes, incorporation of a thiophosphorylated building block would be a superior alternative. Due to the limitations of Boc-methodology we have found it desirable to develop alternative synthetic strategies, which would complement the existing methods allowing greater flexibility in the synthesis of chemically diverse collections of compounds containing thiophosphorylated aminoacids.

The *O,O*-diallyl-thiophosphorylated *N*-Alloc, *N*-Fmoc and *N*-Boc protected serine, **4**-**9** were prepared in a one pot procedure from the corresponding *N*-protected amino acids.

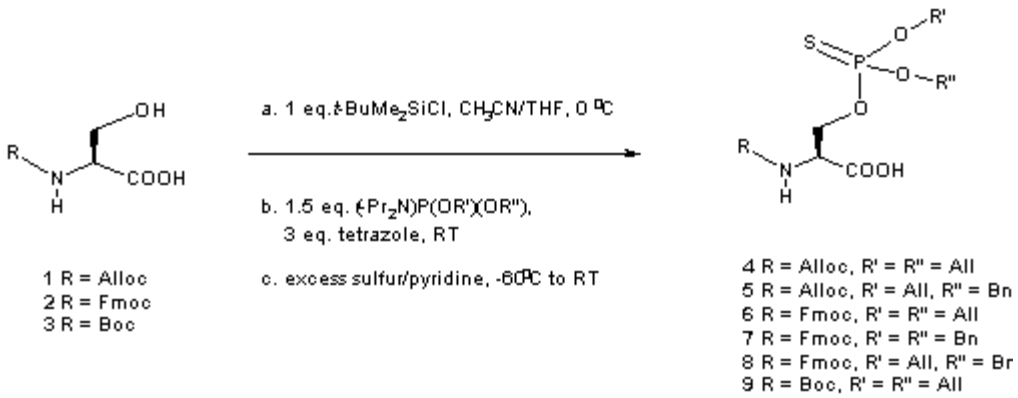
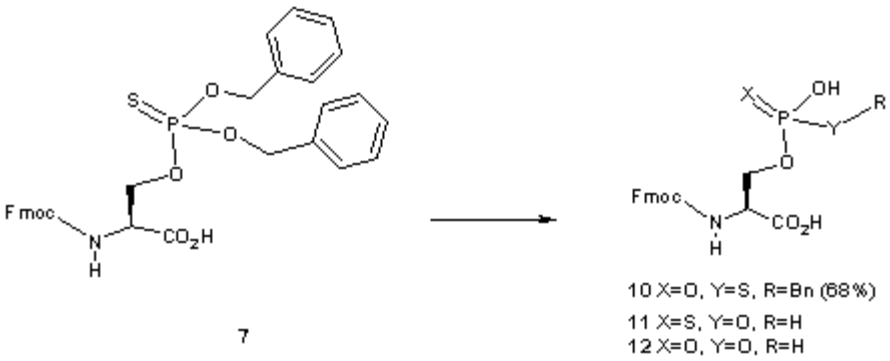


Table 1. Properties of the Protected Thiophosphate

Compound	yield	$[a]_D^{20}$ (CH_2Cl_2)	^{31}PNMR (d)	mp
4 *N*-Alloc-*O,O*-diallylthiophosphoserine	46%	+ 9.4 (c= 1.2)	69.8[a]	yellow oil
5 *N*-Alloc-*O*-allyl-*O*-benzylthiophosphoserine	46%		68.2, 68.2[b]	yellow oil[d]
6 *N*-Fmoc-*O,O*-diallylthiophosphoserine	38%	+11.7 (c=0.3)	76.5[b]	72-75 ^{0}C
7 *N*-Fmoc-*O,O*-dibenzylthiophosphoserine	43%	+ 5.9 (c=1.1)	68.1[b]	147-148 ^{0}C
8 *N*-Fmoc-*O*-allyl-*O*-benzylthiophosphoserine	32%		68.2, 68.3[b]	yellow oil
9 *N*-Boc-*O,O*-diallylthiophosphoserine	11%[c]	+ 7.4 (c=1.1)	95.4[b]	yellow oil

[a] $CDCl_3$ [b] DMSO-d_6 [c] One equivalent of diallyl phosphoramidite used.
[d] 1:1 mixture of diastereoisomers at P.

The *N*-Fmoc-*O,O*-dibenzyl-thiophosphoserine 7, can be converted to *N*-Fmoc-*S*-benzyl-thiophosphoserine 10 in an acid-catalysed rearrangement. In addition to being less prone to base-catalysed [beta]-elimination of the thiophosphate moiety than the corresponding triesters, the protected phosphodiester 10 has the additional advantage of blocking the nucleophilic sulfur of the thiophosphate. Under various conditions (see table 2 below), either simple *O*-deprotection or hydrolysis of the thiophosphate is observed.

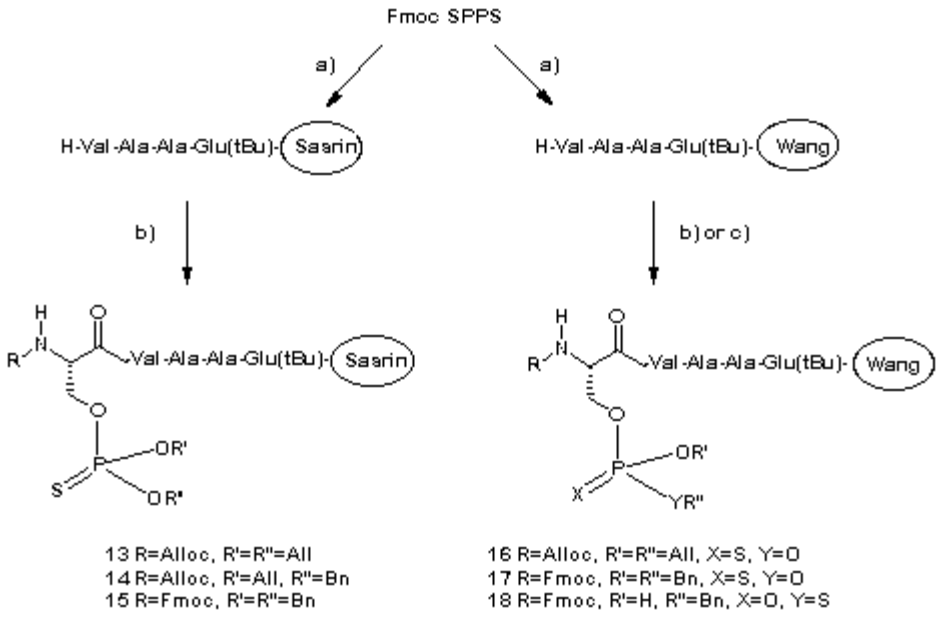


7

Table 2 Conditions for Benzyl Deprotection of 7

Reaction conditions	Products	Yield	Notes
TMSBr (3 eq), TFA, r.t., 1 h	**10**[a]	68%	[a] a= 24.0 (c= 0.5), ^{31}P-NMR= 25.1
TMSBr (3 eq), TFA (1 eq), CH_2Cl_2, r.t., 24 h	**7**[b]		[b] Traces of **10** (by TLC)
TFA, 2.5% H_2O, 2.5% C_6H_5SH. [4]	**10** + **11**+ **12** [c]		[c] By ^{31}P-NMR and HPLC
TFA, 2.5% H_2O	**10** + **11**	67%, 23% [d]	[d] Determination by HPLC
TMSBr,TFA, *m*-cresol, C_6H_5SH.[5]	**11**	89% [e]	[e] Determinaton by HPLC
NaI(2 eq), CH_3CN/THF (1:1), r.t., 24 h.[6]	**7**[f]		[f] By TLC

After solid-phase peptide synthesis, building blocks 4, 5, 7 and 10 provide direct access to *N*-protected-*N*-terminal *O*-thiophosphorylated peptides.



a) Fmoc-Amino Acids , tBu= t-butyl; Amino acid couplings 3eq. DIC-HOBt-FmocAA (1:1:1) preactivated in DMF; Fmoc cleavage: Piperidine-DMF (20%). b) 2 x 3eq. R-Ser[PS(OR')(OR")]-OH/DIC/HOBt (1:1:1) DMF, 3h, RT. c) 3eq. Fmoc-Ser[PO(OH)(SBn)]-OH/HBTU/DIEA (1:3:3) DMF, 3h, RT.

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Comments

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