

**DI-*t*-BUTYL *N,N*-DIETHYLPHOSPHORAMIDITE AND DIBENZYL *N,N*-DIETHYLPHOSPHORAMIDITE.
 HIGHLY REACTIVE REAGENTS FOR THE 'PHOSPHITE-TRIESTER'
 PHOSPHORYLATION OF SERINE-CONTAINING PEPTIDES**

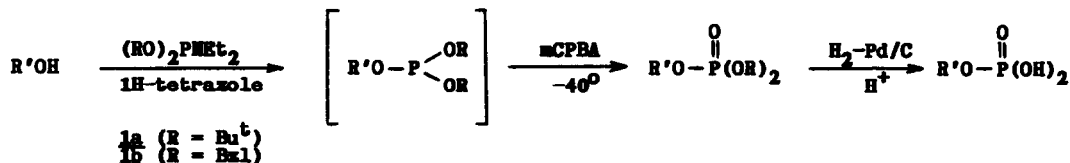
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ABSTRACT: *Di-*t*-butyl *N,N*-diethylphosphoramidite and dibenzyl *N,N*-diethylphosphoramidite were found to be highly reactive reagents for the efficient 'phosphite-triester' phosphorylation of protected serine derivatives (Boc-Ser-ONBzl), protected serine-containing peptides and resin-bound protected serine-containing peptides.*

As an alternative to the use of dibenzyl and di-*t*-butyl phosphorohalidates for the phosphorylation of alcohols,¹⁻³ our recent studies have concentrated on the use of dialkyl *N,N*-diethylphosphoramidites for the efficient phosphorylation of alcohols and hydroxy-containing biomolecules. In particular, we have concentrated on the use of benzyl and *t*-butyl groups for temporary phosphate protection on account of the facile hydrogenolytic¹ or acidolytic³ cleavage of these phosphate protecting groups. From these studies, we have demonstrated that the phosphoramidite reagents, di-*t*-butyl *N,N*-diethylphosphoramidite **1a** and dibenzyl *N,N*-diethylphosphoramidite **1b**, are stable yet highly reactive reagents for the 'phosphite-triester' phosphorylation of simple alkyl alcohols [(i) (RO)₂PNet₂ (R = Bu^t or Bzl)/1H-tetrazole, (ii) mCPBA] and give the respective di-*t*-butyl and dibenzyl phosphorotriesters in 90-99% yields (Scheme 1).^{4,5} In this letter, we report the use of

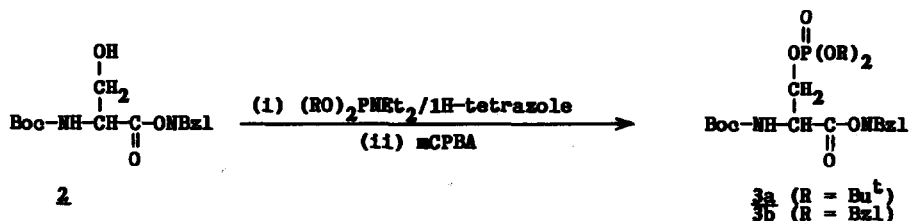
Scheme 1.



1a and **1b** for the efficient 'phosphite-triester' phosphorylation of a protected serine and tyrosine derivative, a protected serine-containing peptide and a resin-bound protected serine-containing peptide.

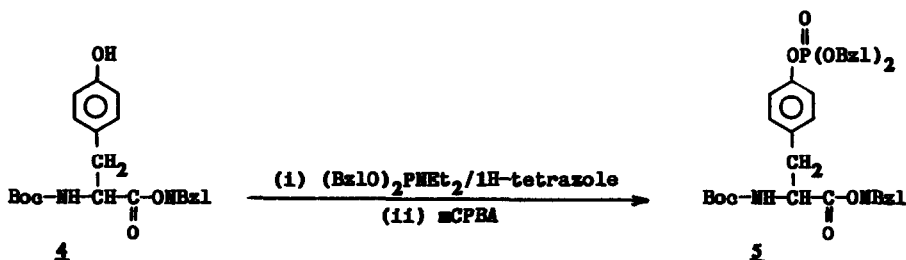
In the first instance, the 'phosphite-triester' phosphorylation procedure was found to be useful for the phosphorylation of simple protected serine derivatives, **1a** or **1b**/1H-tetrazole treatment of Boc-Ser-ONBzl **2** followed by *in situ* non-nucleophilic oxidation (mCPBA) affording Boc-Ser(PO₃Bu^t)₂-ONBzl **3a** and Boc-Ser(PO₃Bzl₂)₂-ONBzl **3b** in 92 and 98% yields respectively (Scheme 2). Products **3a** and **3b** gave typical ³¹P NMR chemical shift values of -9.6 and +0.8 ppm respectively and their structures were confirmed by the observation of phosphorus-coupled doublet signals for the C_α and C_β carbons of the seryl residue (J_{PC} 5.8 Hz) in their ¹³C NMR spectra.

Scheme 2.



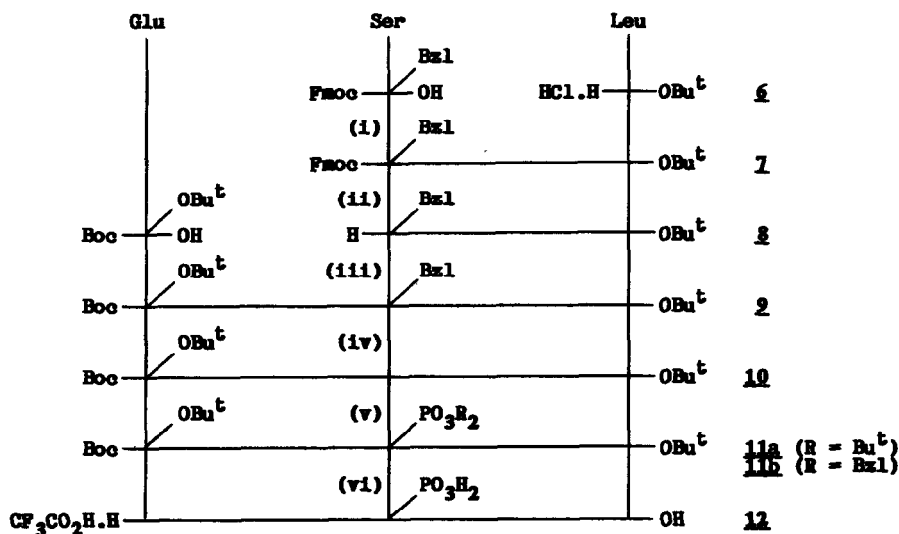
Alternatively, this procedure can also be applied to the phosphorylation of protected tyrosine derivatives, the phosphitylation of Boc-Tyr-ONBzl using **1b**/1H-tetrazole followed by mCPBA oxidation giving Boc-Tyr(PO₃Bzl₂)₂-ONBzl **5** in near-quantitative yield (Scheme 3). This procedure is far superior to other reported procedures in that it does not require pre-generation of the sodium phenoxide⁷ or suffer from the low reactivity of the dibenzyl hydrogen phosphonate/carbon tetrachloride phosphorylation procedure.⁸

Scheme 3.



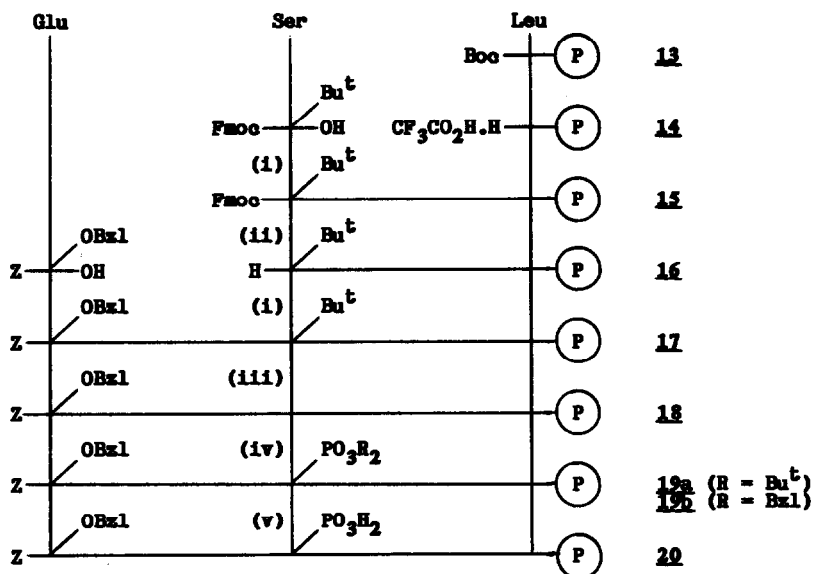
In addition, the 'phosphite-triester' phosphorylation procedure was also extended to the phosphorylation of a protected serine tripeptide Boc-Glu(OBu^t)-Ser-Leu-OBu^t. The synthesis of the serine-containing peptide **10** was accomplished by the solution phase incorporation of Fmoc-Ser(Bu^t)-OH using the mixed anhydride coupling procedure, the use of 10% diethylamine/DMF⁹ for removal of the Fmoc group from dipeptide **7** and final hydrogenolytic removal of the seryl benzyl ether group. The phosphorylation of **10** in dry THF using either **1a** or **1b** proceeded smoothly and gave Boc-Glu(OBu^t)-Ser(PO₃Bu^t)₂-Leu-OBu^t **11a** or Boc-Glu(OBu^t)-Ser(PO₃Bzl₂)₂-Leu-OBu^t **11b** in 95 and 96% yields respectively after purification. The structural characterization of these products was readily established by their ¹³C NMR spectra^{10,11} and ³¹P NMR spectra, ³¹P NMR values of -9.2 and -0.8 ppm being observed for **11a** and **11b** respectively. Subsequent hydrogenolytic or acidolytic treatment of **11a** and **11b** respectively in 40% TFA/AcOH effected ready removal of the protecting groups and gave the pSer-tripeptide CF₃CO₂H-H-Glu-Ser(PO₃H₂)-Leu-OH in quantitative yield. Structural confirmation and the purity of **12** was established from its ³¹P NMR spectrum (+0.10 ppm), its

^{13}C NMR spectrum and from its FAB-mass spectrum (Ar, +ve mode, m/z 348, M^+). Although PSer-containing peptides have previously been prepared via the use of temporary benzyl phosphate protection, the synthesis of **12** from **11a** represents the first PSer-peptide that has been prepared via the use of *t*-butyl phosphate protection. A feature in the use of *t*-butyl phosphate protection is that the di-*t*-butyl phosphotriester functionality is readily deprotected with the use of mild acid treatments and that the isolation of the PSer-containing peptide is simple and effected in a metal-free (Ca^{2+}) environment.



- (i) Et_3N (1 eq) then $\text{Et}_3\text{N}/\text{IBCF}$ (-20° , 2h), (ii) 10% $\text{Et}_3\text{N}/\text{DMF}$ (20° , 2h)
 (iii) $\text{Et}_3\text{N}/\text{IBCF}$ (-20° , 2 h), (iv) H_2 -Pd/C, AcOH.
 (v) $(\text{RO})_2\text{PNEt}_2$ (R = Bu^t or Bzl)/1H-tetrazole (10 min), then mCPBA (-40° , 5 min),
 (vi) 40% TFA/AcOH (R = Bu^t) or H_2 -Pd/C, 40% TFA/AcOH (R = Bzl).

In continuation of this study, both **1a** and **1b** were also suitable for the 'phosphite-triester' phosphorylation of the resin-bound seryl-containing tripeptide, Z-Glu(OBzl)-Ser-Leu- P (P = 1% cross-linked polystyrene resin). The resin-bound tripeptide was prepared using Merrifield 1% cross-linked polystyrene resin as the peptide support, the incorporation of Fmoc-Ser(Bu^t)-OH into peptide synthesis (DCC/HOBt) using the Merrifield peptide synthesis protocol, 20% piperidine/DMF for removal of the Fmoc group from **15** and 90% TFA/ CH_2Cl_2 for acidolytic removal of the seryl *t*-butyl ether group. The phosphorylation of **18** using either **1a** or **1b** proceeded quantitatively and was confirmed by analysis of the ^{13}C NMR and ^{31}P NMR spectra obtained from the peptide-resins swollen in CDCl_3 , these latter spectra giving typical chemical shift values for **19a** and **19b** of -9.2 and -0.3 ppm respectively. In the case of **19a**, further treatment of the peptide-resin with 10% TFA/ CH_2Cl_2 readily removed the *t*-butyl groups and gave the protected resin-bound Ser(PO_3H_2)-containing peptide **20**, the ^{31}P NMR spectrum of **20** showing a broad single peak at 0.0 ppm. These results clearly show that the 'phosphite-triester' phosphorylation procedure can be extended to resin-bound serine-containing peptides and, in the case of *t*-butyl protection, the di-*t*-butyl phosphates can be readily converted to their mono-alkyl phosphates.



(i) DCC/HOBt (20°, 3 h), (ii) 20% piperidine/DMF (1x3 min, 1x7 min),

(iii) 90% TFA/CH₂Cl₂ (60 min), (iv) (RO)₂PNET₂ (R = Bu^t or Bzl)/1H-tetrazole (15 min) then mCPBA (10 min), (v) 10% TFA/CH₂Cl₂ (R = Bu^t) (30 min).

This work clearly demonstrates that **1a** and **1b** are ideal reagents for the 'phosphite-triester' phosphorylation of protected serine derivatives and the phosphorylation of serine-containing peptides. In view of the ready removal of *t*-butyl and benzyl phosphate groups, we consider the phosphoramidite-based phosphorylation procedure described here will find wide application in the general phosphorylation of other simple serine-containing peptides and hydroxy-containing biomolecules.

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6. ¹³C NMR (CDCl₃) **5**: δ 28.3, 37.3, 55.1, 65.4, 70.0 (d, 4.4 Hz), 80.0, 120.2 (d, 5.9 Hz), 123.7, 128.0, 128.5, 128.7, 130.6, 133.3, 135.4 (d, 7.3 Hz), 142.6, 147.6, 149.6 (d, 5.9 Hz), 155.4, 171.7.
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10. ¹³C NMR (CDCl₃) **11a**: δ 22.0, 22.6, 24.6, 27.6, 27.8, 27.9, 28.2, 29.7 (d, J_{PC} 3.3 Hz), 31.9, 41.3, 51.7, 53.2 (d, 5.5 Hz), 54.8, 63.8 (d, 5.5 Hz), 79.0, 80.7, 81.4, 83.2 (d, 7.7 Hz), 155.5, 168.1, 171.0, 172.0, 172.4, 174.8.
11. ¹³C NMR (CDCl₃) **11b**: δ 21.8, 22.7, 24.7, 27.4, 27.8, 27.9, 28.2, 31.9, 41.2, 51.7, 53.1 (d, 5.9 Hz), 54.8, 66.7 (d, 5.9 Hz), 79.0, 80.7, 81.4, 155.5, 168.1, 171.0, 172.0, 172.4, 174.7.

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