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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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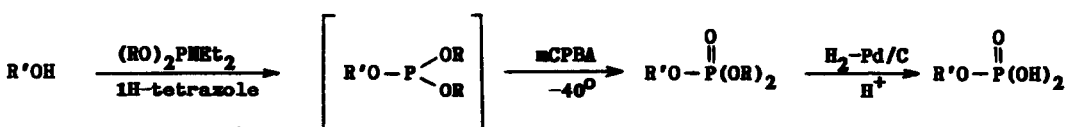
ABSTRACT: Di-t-butyl N,N-diethylphosphoramidite and dibenzyl N,N-diethylphosphoramidite were found to be highly reactive reagents for the efficient 'phosphite-triester' phosphorylation of protected serine derivatives (Boc-Ser-ONBzl), protected serine-containing peptides and resin-bound protected serine-containing 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 hydrogenolytic[1] or acidolytic[3] 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) $(RO)_2PNEt_2$ (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.

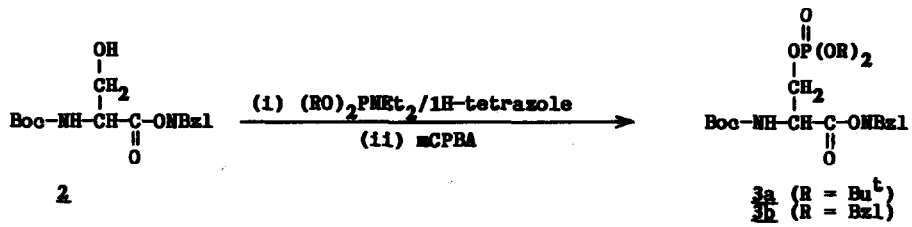


1a (R = Bu^t)
1b (R = Bzl)

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($PO_3Bu^t_2$)-ONBzl 3a and Boc-Ser(PO_3Bzl_2)-ONBzl 3b in 92 and 98% yields respectively (Scheme 2). Products 3a and 3b gave typical ^{31}P 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 ^{13}C NMR spectra.

Scheme 2.



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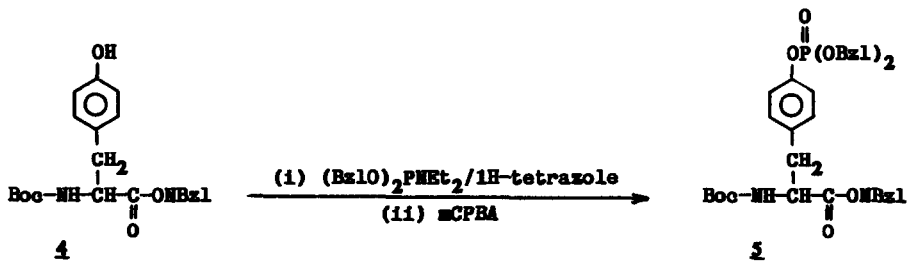
3a (R = Bu^t)
3b (R = Bzl)

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(PO_3Bzl_2)-ONBzl[6] 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 phenoxide[7] or suffer from the low reactivity of the dibenzyl hydrogen phosphonate/carbon tetrachloride phosphorylation procedure.[8]

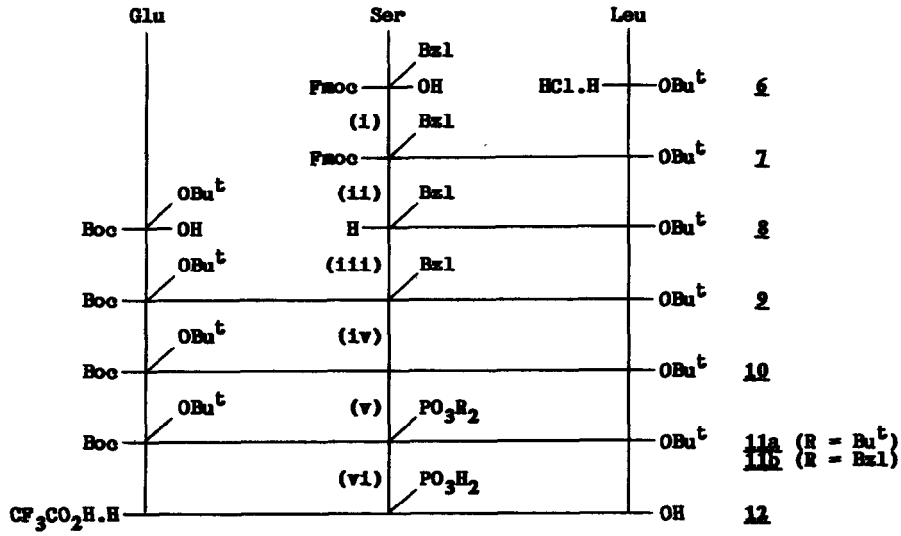
Scheme 3.



4 5

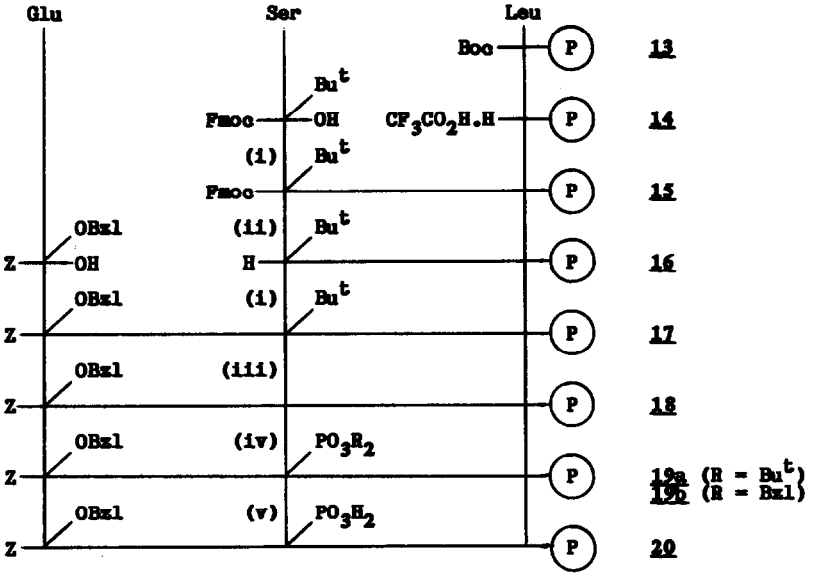
In addition, the 'phosphite-triester' phosphorylation procedure was also extended to the phosphorylation of a protected serine tripeptide Boc-Glu(OBu^t)-Ser-Leu-OBu^t. The synthesis of the serine-containing peptide 10 was accomplished by the solution phase incorporation of Fmoc-Ser(Bu^t)-OH using the mixed anhydride coupling procedure, the use of 10% diethylamine/DMF[9] 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($PO_3Bu^t_2$)-Leu-OBu^t 11a or Boc-Glu(OBu^t)-Ser(PO_3Bzl_2)-Leu-OBu^t 11b in 95 and 96% yields respectively after purification. The structural characterization of these products was readily established by their ^{13}C NMR spectra[10,11] and ^{31}P NMR spectra, ^{31}P 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 CF_3CO_2H·H-Glu-Ser(PO_3H_2)-Leu-OH in quantitative yield. Structural confirmation and the purity of 12 was established from its ^{31}P 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 phosphorotriester 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) NMM (1 eq) then NMM/IBCF (-20°, 2h), (ii) 10% Et_2NH/DMF (20°, 2h)
(iii) NMM/IBCF (-20°, 2 h), (iv) H_2-Pd/C, AcOH,
(v) $(RO)_2PNEt_2$ (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/CH_2Cl_2 (60 min), (iv) $(RO)_2PNEt_2$ (R = Bu^t or Bzl)/1H-tetrazole (15 min) then mCPBA (10 min), (v) 10% TFA/CH_2Cl_2 (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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REFERENCES AND NOTES

1. Folsch, G., **Sven. Kem. Tidskr.**, **79**, 38, (1967).

2. Khwaja, T. A., Reese, C. B. and Stewart, J. C. M., **J. Chem. Soc. (C)**, 2092, (1970).

3. Gajda, T. and Zwierzak, A., **Synthesis**, 623, (1978).

4. Perich, J. W. and Johns, R. B., **Tetrahedron Lett.**, 101, (1987).

5. Perich, J. W. and Johns, R. B., **Synthesis**, 142, (1988).

6. ^{13}C NMR ($CDCl_3$) 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.

7. Valerio, R. M, Alewood, P. F., Johns, R. B., Kemp, B. E., **Tetrahedron Lett.**, 2609, (1984).

8. Gibson, B. W., Falick, A. M., Burlingame, A. L., Nadasdi, L., Nguyen, A. C. and Kenyon, G. L., **J. Am. Chem. Soc.**, **109**, 5343, (1987).

9. Bodanszky, A., Bodanszky, M., Chandramouli, N., Kwei, J. Z., Martinez, J., and Tolle, J. C., **J. Org. Chem.**, **42**, 72, (1980).

10. ^{13}C NMR ($CDCl_3$) 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, 65.8 (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. ^{13}C NMR ($CDCl_3$) 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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