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# SOLID-PHASE SYNTHESIS OF AN O-PHOSPHOSERYL-CONTAINING PEPTIDE USING PHENYL PHOSPHOROTRIESTER PROTECTION

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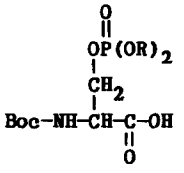
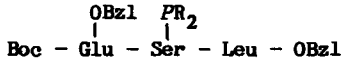
*ABSTRACT: The solid-phase synthesis of TFA · $H_2$-Glu-PSer-Leu* 1 *is reported via incorporation of the protected phosphoroamino acid* $N_\alpha$*-tert-butoxycarbonyl-O-(diphenylphosphoro)-L-serine* 2b.

In our earlier studies [1,2], we reported the solution-phase synthesis of Glu-*P*Ser-Leu via a novel strategy which specifically incorporated a protected O-phosphoroserine derivative into a peptide sequence, thereby, uniquely determining the position of the O-phosphoseryl residue. This strategy involved the initial synthesis of the protected tripeptides 6a and 6b using 2a[3] and 2b[2,4] respectively followed by the hydrogenolytic removal of the benzyl or phenyl phosphorotriester protecting groups.



2 a) R = Bzl
b) R = Ph



6 a) R = Bzl
b) R = Ph

We now report the use of 2b[5] in the synthesis of TFA · $H_2$-Glu-*P*Ser-Leu 1 via Merrifield solid-phase methodology[6]. By successive DCCI-HOBt couplings[6] of Boc-Ser[*P*$Ph_2$]-OH 2b and Boc-Glu[OBzl]-OH 4 with 1.2g of Boc-Leu-Resin 3[7] (1.0 mmol/g) and 40% TFA/$CH_2Cl_2$ for cleavage of the Boc group from intermediate resin-bound peptides, 1.7g of resin-bound peptide 5 was obtained.

The $^{13}C$ nmr spectrum of 5 in $CDCl_3$[8] (shown in Fig. 1) was found to be markedly similar to the $^{13}C$ nmr spectrum obtained for Boc-Glu[OBzl]-Ser[$PPh_2$]-Leu-OBzl 6b[2] (Fig. 2) and displayed the characteristic phosphorus-coupled doublet signals for the α- and β-carbons of the O-(diphenylphosphoro)seryl residue at 53.3 and 67.8 ppm (each $J_{PC}$ 5.9 Hz). The $^{31}P$ nmr spectrum of 5 contained a single broad resonance at -11.7 ppm[9] and compared favourably with a value of -12.1 ppm[9] obtained for 6b[2].

Hydrogenolysis of 5 (1.20g) in 40% TFA/DMF[10] with $Pd(OAc)_2$[11] simultaneously cleaved the peptide from the resin and removed the glutamyl benzyl and N-terminal Boc protecting groups[12]. A final hydrogenolytic treatment of the crude O-(diphenylphosphoro)tripeptide 7 in 40% TFA/AcOH with $PtO_2$ (1.0 eq/phenyl group) cleaved the phenyl phosphorotriester groups and gave 1 (250 mg) as a white solid after trituration with diethyl ether. Chromatographic purification of 1[13] was not required since $C_{18}$ RP-HPLC analysis (μ Bondapak C18, 0-50% $CH_3CN$/0.1% TEAP pH 4.0, 1 ml/min, 214 nm) showed it to be homogeneous (> 95% pure)[14]. In addition to amino acid analysis of the acid hydolysate of 1 giving the constituent amino acid residues in the expected molar ratios ($Glu_1$ 1.00, $Ser_1$ 0.95, $Leu_1$ 0.95), chiral evaluation of these amino acid residues via $C_{18}$ RP-HPLC analysis of their L-Leucyl dipeptides established them to be racemization free.

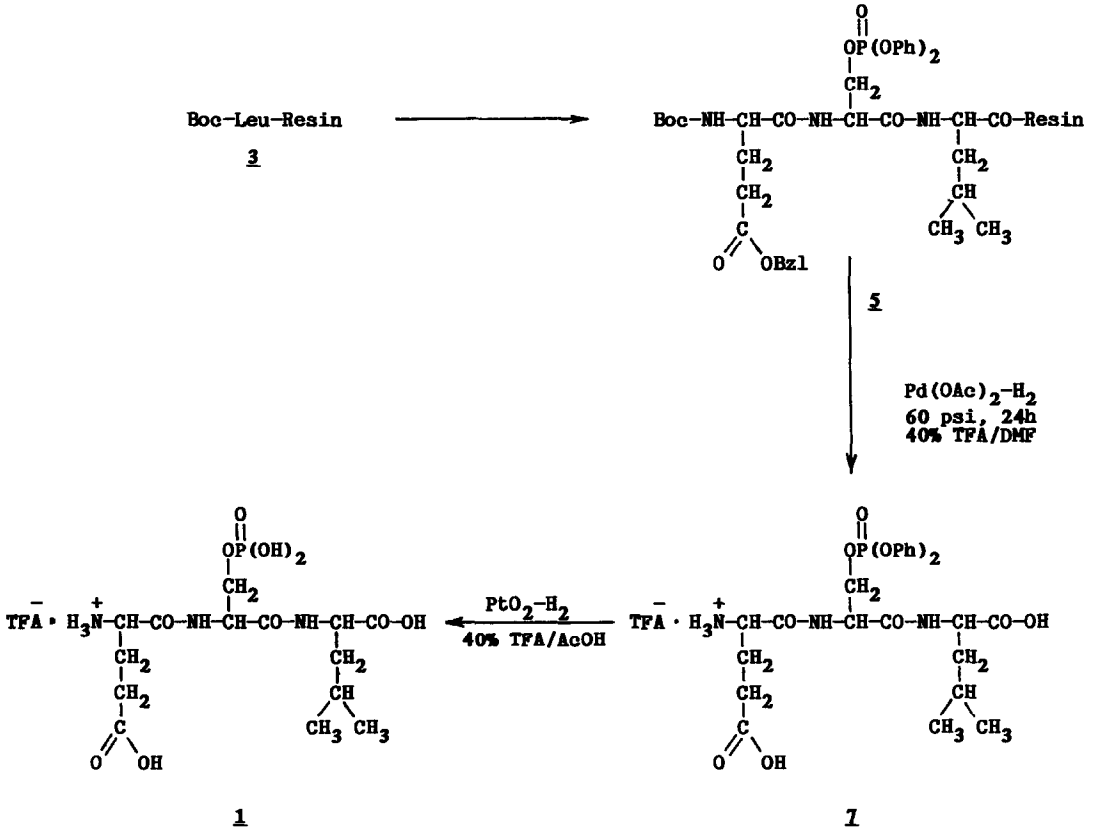


Fig. 1

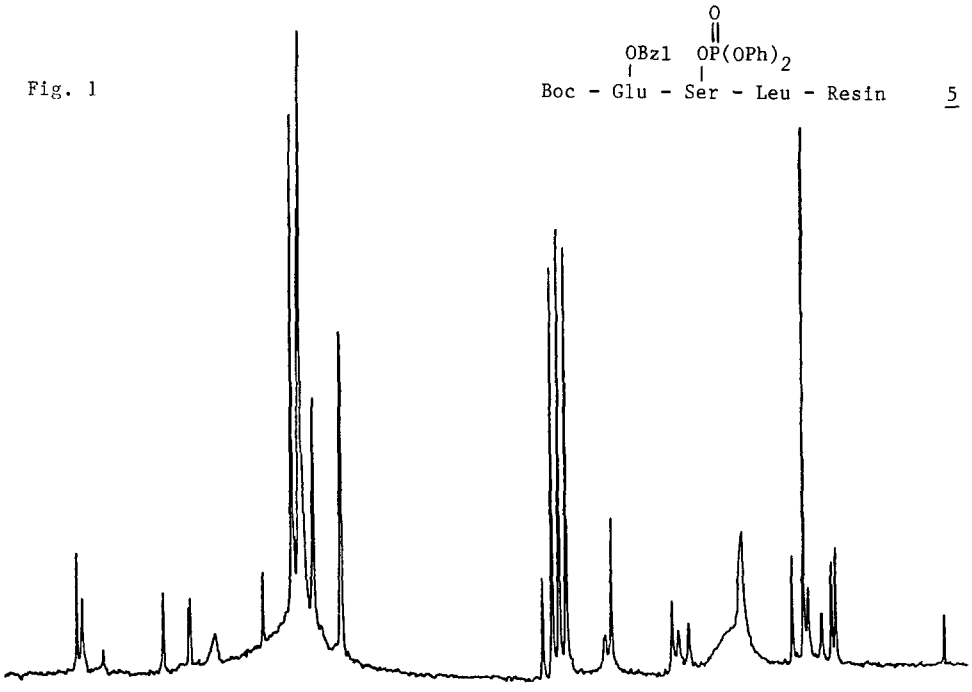
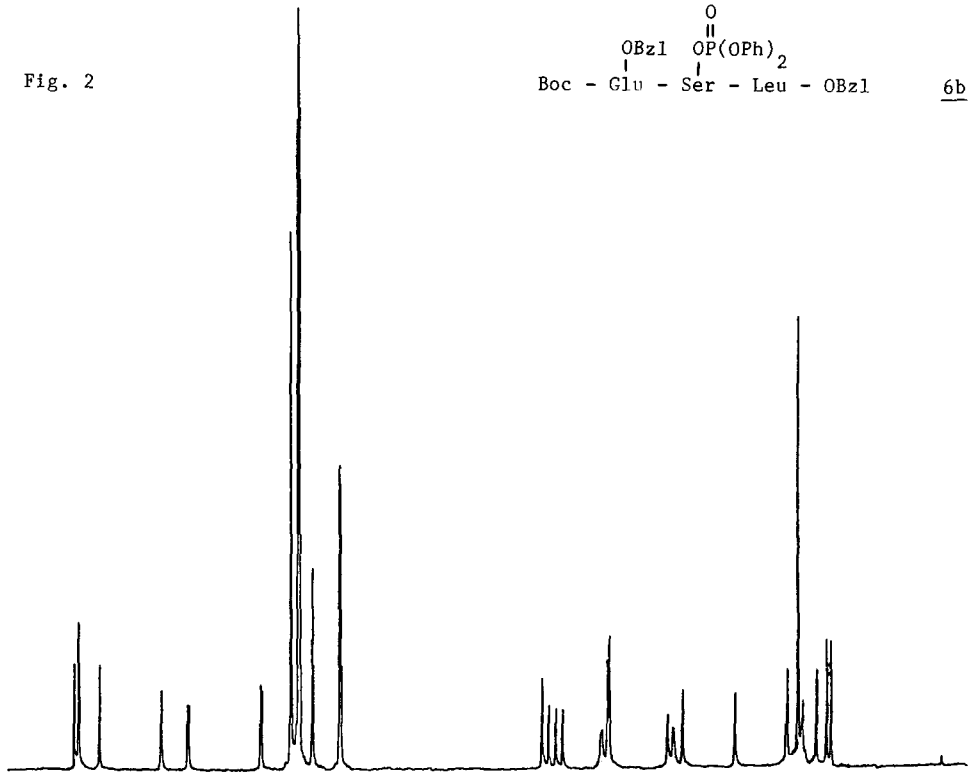

O
OBzl OP(OPh)2
Boc - Glu - Ser - Leu - Resin 5

Fig. 2


O
OBzl OP(OPh)2
Boc - Glu - Ser - Leu - OBzl 6b

The efficient synthesis of TFA · $H_2$-Glu-*P*Ser-Leu **1** using Boc-Ser[*P*$Ph_2$]-OH **2b** demonstrates that N-protected O-(disubstitutedphosphoro)serine derivatives are readily adapted to solid-phase peptide synthesis and indicates that the synthesis of longer and more complex O-phosphoserine-containing peptides to be a practical proposition.

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NOTES AND REFERENCES:

1. P.F. Alewood, J.W. Perich and R.B. Johns, Tetrahedron Lett., 25, 987, (1984).
2. J.W. Perich, P.F. Alewood and R.B. Johns, Tetrahedron Lett., preceeding publication.
3. P.F. Alewood, J.W. Perich and R.B. Johns, Aust. J. Chem., 37, 429, (1984).
4. J.W. Perich, P.F. Alewood and R.B. Johns, Synthesis, accepted for publication (1985).
5. **2a** could not be satisfactorily applied to solid-phase synthesis due to the acidolytic sensitivity of benzyl phosphorotriester groups to 40% TFA/$CH_2Cl_2$.
6. R.S. Hodges and R.B. Merrifield, Anal. Biochem., 65, 241, (1975).
7. Prepared by the esterification of Boc-Leu-$O^-$ $^+Cs$ with chloromethylated styrene-1% divinylbenzene resin, 1.04 mmol Cl/g resin (FLUKA).
8. $^{13}C$ nmr δ ($CDCl_3$) **5** : 21.9, 22.7, 24.7, 27.4, 28.3, 30.6, 40.6, 51.2, 53.3 (d, $J_{PC}$ = 5.9 Hz), 57.5, 66.6, 67.8 (d, J = 5.9 Hz), 80.34, 120.1 (d, $J_{PC}$ = 4.9 Hz), 125.6, 128.2, 128.6, 129.9, 135.8, 150.3 (d, $J_{PC}$ = 7.3 Hz), 167.7, 171.9 and 173.0 ppm.
9. Relative to ext. 85% $H_3PO_4$.
10. Hydrogenolysis of **5** in neat DMF gave rise to an unknown side-product.
11. J.M. Schlatter, R.H. Mazur and O. Goodmonson, Tetrahedron Lett., 23, 2851, (1977).
12. Under these conditions, $^{31}P$ nmr spectroscopy showed ~5% cleavage of a phenyl group occurred.
13. Identical in all respects with TFA· Glu-*P*Ser-Leu prepared via solution-phase peptide synthesis[2].
14. $C_{18}$ RP-HPLC analysis of **1** confirmed quantitative incorporation of **2b** had occurred during peptide synthesis since it was not contaminated with either TFA · *P*Ser-Leu or leucine.

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