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

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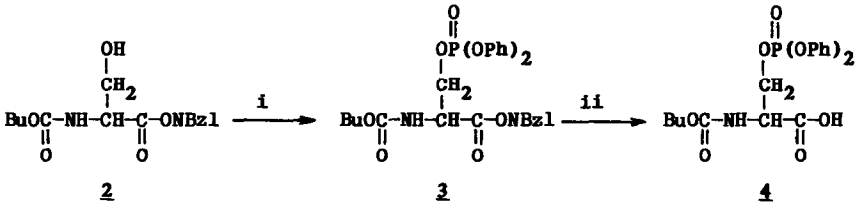
*ABSTRACT: The solution-phase synthesis of TFA · $H_2$-Glu-PSer-Leu 9 is reported using the protected phosphoroamino acid, $N_\alpha$-tert-butoxycarbonyl-O-(diphenylphosphoro)-L-serine 4.*

In our previous communication[1], we reported the synthesis of Glu-*P*Ser-Leu **1** using a protected O-(dibenzylphosphoro)serine derivative. This synthesis involved initial preparation of the protected tripeptide Boc-Glu[OBzl]-Ser[*P*$Bzl_2$]-Leu-OBzl using Boc-Ser[*P*$Bzl_2$]-OH [2] followed by hydrogenolytic removal of benzyl protecting groups. However, in this instance, peptide synthesis was complicated due to partial acidolytic debenzylation of the dibenzylphosphorotriester functionality during cleavage of the N-terminal Boc group from Boc-Ser[*P*$Bzl_2$]-Leu-OBzl with 40% TFA/$CH_2Cl_2$ or 4M HCl/Dioxane. Although debenzylation was minimized by the use of formic acid, this inherent difficulty complicated the synthesis of longer and more complex O-(dibenzylphosphoro)seryl-containing peptides.

In order to overcome this synthetic difficulty, we therefore investigated phenyl protection of the phosphate functionality. The synthesis of Boc-Ser[*P*$Ph_2$]-OH **4** is outlined in Scheme 1 and involved initial phosphorylation of **2** with $(PhO)_2POCl$/pyridine followed by hydrogenolytic cleavage of the 4-nitrobenzyl group from **3**. Whilst the characterization of **2** and **4** are described elsewhere[3,4], the phosphorylated intermediate **3** was readily characterized[5] and gave consistent $^1H$, $^{13}C$ and $^{31}P$ nmr spectra[6].

**Scheme 1**

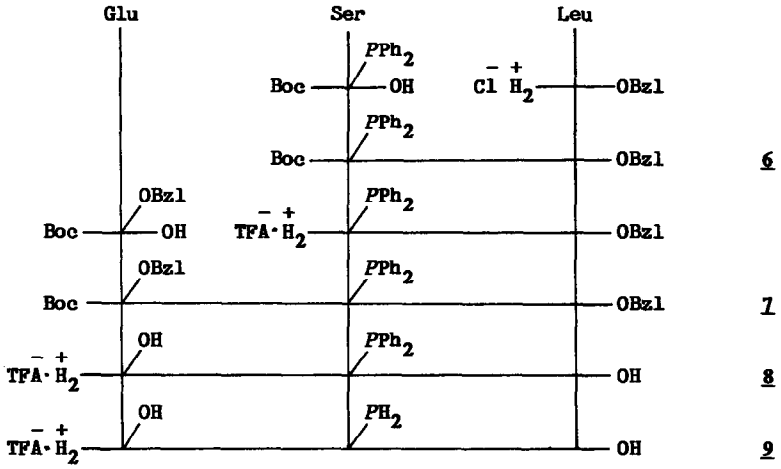



**2** **3** **4**

**(i)** $(PhO)_2POCl$/pyridine **(ii)** Pd/C-$H_2$

The synthesis of TFA · $H_2$-Glu-Ser[$PPh_2$]-Leu 8 using Boc-Ser[$PPh_2$]-OH 4 is outlined in Scheme 2. While the intermediate protected di- and tri-peptides 6 and 7 were obtained in 90 and 96% yields[7] respectively using the excess mixed anhydride coupling procedure[8] and 40% TFA/$CH_2Cl_2$ (or 4M HCl/dioxane) for the cleavage of the Boc group from 6, the O-(diphenylphosphoro)tripeptide 8 was obtained in quantitative yield by hydrogenolysis of 7 in 40% TFA/AcOH. Peptides 6, 7 and 8 were homogeneous by TLC criteria, were readily characterized by their $^{13}C$ nmr spectra[9,10,11] and had $^{31}P$ nmr values of -12.0, -12.1 and -12.9 ppm[12] respectively. Although a $^{31}P$ nmr spectroscopy study confirmed that phenyl phosphorotriester groups were stable to 40% TFA/$CH_2Cl_2$ for prolonged periods, we found that the O-(diphenylphosphoro)seryl residue was unstable in 45% HBr/AcOH and that diphenylphosphonic acid was completely cleaved from 8 after 5 days ($t_{½}$ ~20h, 20°)[13].

**Scheme 2**




Initial attempts at hydrogenolytic cleavage of phenyl groups from 8 in methanol using 0.1 eq. $PtO_2$/phenyl group[14] resulted in incomplete hydrogenolysis. However, by changing to 1.0 eq. $PtO_2$/phenyl group and 40% TFA/AcOH as solvent, hydrogenolysis proceeded rapidly and was complete within 15 mins. Removal of catalyst and evaporation of solvent gave 9 as a white solid in quantitative yield. The rapid consumption of hydrogen was unexpected since this process has generally been reported in the literature to be slow (up to 8h) and incomplete[15]. Indeed, we found that hydrogenolysis required up to 12h if the quantity of platinum catalyst was reduced to 0.5 eq. $PtO_2$/phenyl group and was incomplete with the use of 0.25 eq. $PtO_2$/phenyl group.

The HPLC profile (μ Bondapak C18, 0-50% $CH_3CN$/0.1% TEAP pH 4.0, 1ml/min, 214 nm) of 9 showed a single peak while $^{13}C$ and $^{31}P$ nmr spectra[16] were consistent with its structure and contained no additional contaminant resonances. 9 was identical in all respects with TFA · $H_2$-Glu-*P*Ser-Leu prepared using benzyl phosphorotriester protection[17].

A comparison of the $^{13}C$ nmr spectra of TFA · $H_2$-Glu-Ser-Leu **10** in 1M HCl (fig. 1) and **9** in 1M HCl (fig. 2) clearly shows the inductive effect of the phosphorus atom on the α- and β-carbons of the seryl residue and confirms **9** to be free of any non-phosphorylated product contamination. The efficient synthesis of TFA · $H_2$-Glu-*P*Ser-Leu **9** employing phenyl phosphorotriester protection demonstrates that the strategy described in this communication is suitable for the synthesis of longer and more complex O-phosphoserine-containing peptides. The application of this strategy to a solid-phase methodology is described in the following paper.

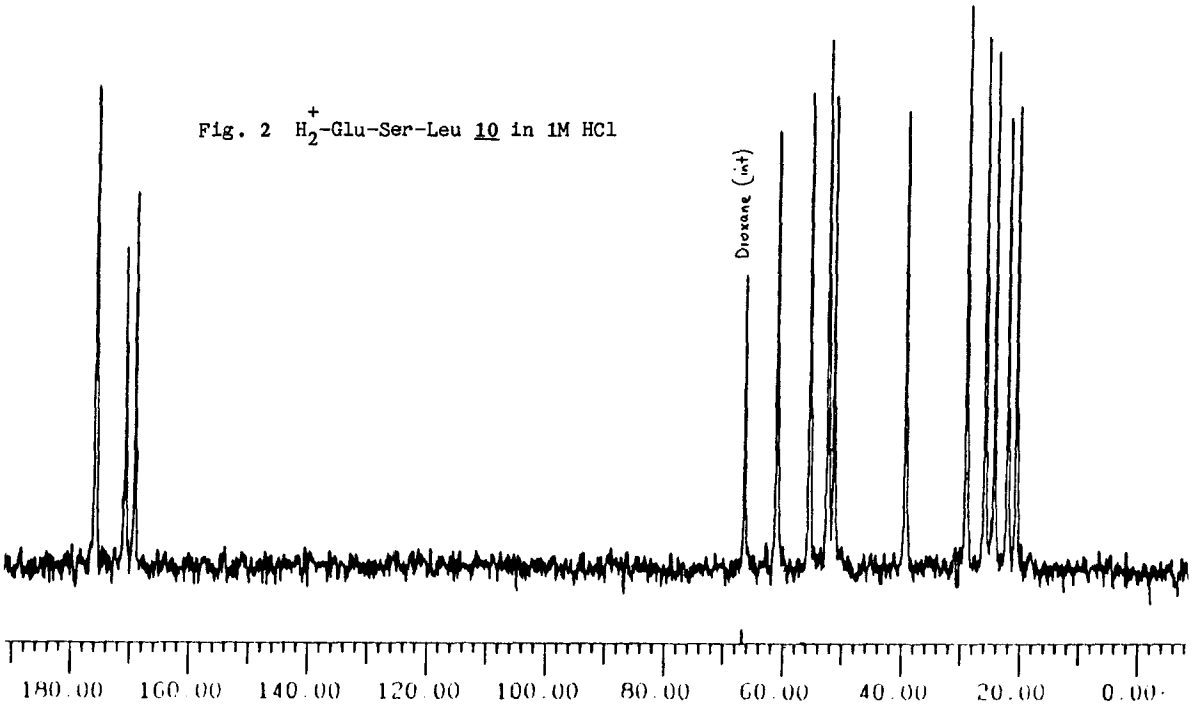



Fig. 2 $H_2^+$-Glu-Ser-Leu **10** in 1M HCl

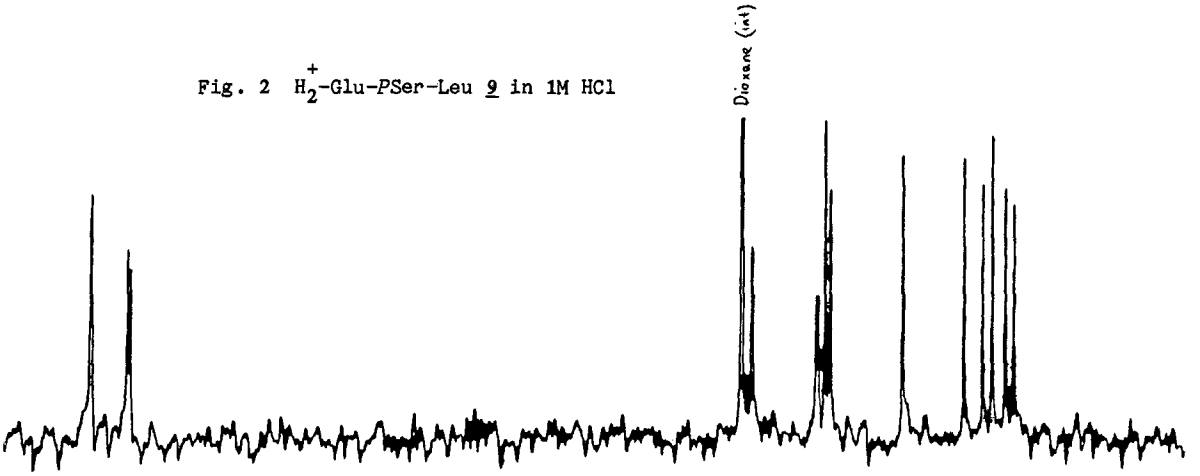



Fig. 2 $H_2^+$-Glu-*P*Ser-Leu **9** in 1M HCl

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NOTES AND REFERENCES

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2. P.F. Alewood, J.W. Perich and R.B. Johns, Aust. J. Chem., 37, 429, (1984).
3. P.F. Alewood, J.W. Perich and R.B. Johns, Synth. Commun., 12, 821, (1982).
4. J.W. Perich, P.F. Alewood and R.B. Johns, Synthesis, accepted for publication (1985).
5. 97% yield, m.p. 113-114.5° (from ethyl acetate/petroleum spirit)
   $[\alpha]^{25}$ +6.71 (c, 1 in $CHCl_3$)
   Found C 56.6, H 5.4, N 4.9, P 5.3%
   Theoretical C 56.6, H 5.1, N 4.9, P 5.4%
6. $^1$H nmr δ ($CDCl_3$) 3: 1.44 (s, 9H, Boc $CH_3$), 4.50-4.80 (m, 3H, Ser α-CH and β-$CH_2$), 5.20 (s, 2H, benzylic $CH_2$), 5.45 (d, 1H, 7.3 Hz, NH), 7.0-7.5 (m, 10H, ArH), 7.44 (d, 2H, J=8.54 Hz, 2-ArH), 8.25 (d, 2H, J=8.54 Hz, 3-ArH).
   $^{13}$C nmr δ ($CDCl_3$) 3: 28.2, 54.0 (d,J=7.3 Hz), 66.0, 68.6 (d,J=5.9 Hz), 80.6, 119.9 (d,J=4.4 Hz), 123.7, 125.6, 128.35, 129.8, 142.0, 147.7, 150.15 (d,J=7.3 Hz), 155.0, 168.7.
   $^{31}$P nmr δ ($CDCl_3$) 3: −12.45 ppm
7. The hydrolysis of excess acylating agent must be conducted below pH 9, otherwise a reduction in yield results.
8. A. von Zon and H.C. Beyerman, Helv. Chim. Acta, 59, 1112, (1976).
9. $^{13}$C nmr δ ($CDCl_3$) 6: 21.9, 22.75, 24.8, 28.2, 41.4, 51.1, 54.6 (d,J=6.1 Hz), 67.1, 68.1 (d,J=6.1 Hz), 80.8, 120.1 (d,J=4.9 Hz), 125.6, 128.2, 128.4, 128.6, 129.9, 135.4, 150.4 (d,J=7.33 Hz), 155.4, 168.4, 172.1.
10. $^{13}$C nmr δ ($CDCl_3$) 7: 21.8, 22.6, 24.65, 27.4, 28.3, 30.4, 40.8, 51.3, 53.2 (d,J=6.1 Hz), 66.4, 66.8, 68.0, (d,J=6.1 Hz), 80.1, 120.05 (d,J=4.9 Hz), 125.5, 128.1, 128.45, 129.15, 129.9, 135.6, 135.8, 150.3 (d,J=7.33 Hz), 155.7, 167.9, 171.95, 172.1, 172.9.
11. $^{13}$C nmr δ ($CD_3OD$) 8: 21.8, 23.3, 25.8, 27.6, 30.0, 40.5, 52.0, 53.5, 54.0 (d,J=6.1 Hz), 68.5 (d,J=6.1 Hz), 121.1 (d,J=4.4 Hz), 126.9, 131.05, 151.5 (d,J=5.9 Hz), 169.4, 169.95, 175.0, 175.5.
12. Relative to ext. 85% $H_3PO_4$
13. P.R. Lashmet, K. Tang and J.K. Coward, Tetrahedron Lett., 24, 1121, (1983).
14. G. Folsch, Svensk Kemisk Tidskrift, 79, 38, (1967).
15. G. Folsch, Acta Chem. Scand., 13, 1407, (1959).
16. $^{13}$C nmr δ ($D_2O$) 9: 20.6, 22.2, 24.4, 25.8, 29.0, 39.3, 51.6, 52.3, 54.2(d,J=7.3 Hz), 64.2(d,J=7.3 Hz), [ 98.9, 110.5, 122.2, 133.8, 160.8, 162.2, 163.6, 165.0, $CF_3COO^-$ ], 169.2, 170.3, 176.1, 176.6.
   $^{31}$P nmr δ ($D_2O$) 9: −0.18 ppm
17. Prepared by treating zwitterionic Glu-*P*Ser-Leu-1 with aqueous TFA.

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