

The Efficient Synthesis of a Complex *O*-Phosphoseryl-containing Peptide Ac-Glu-*P*Ser-Leu-*P*Ser-*P*-Ser-*P*Ser-Glu-Glu-NHMe

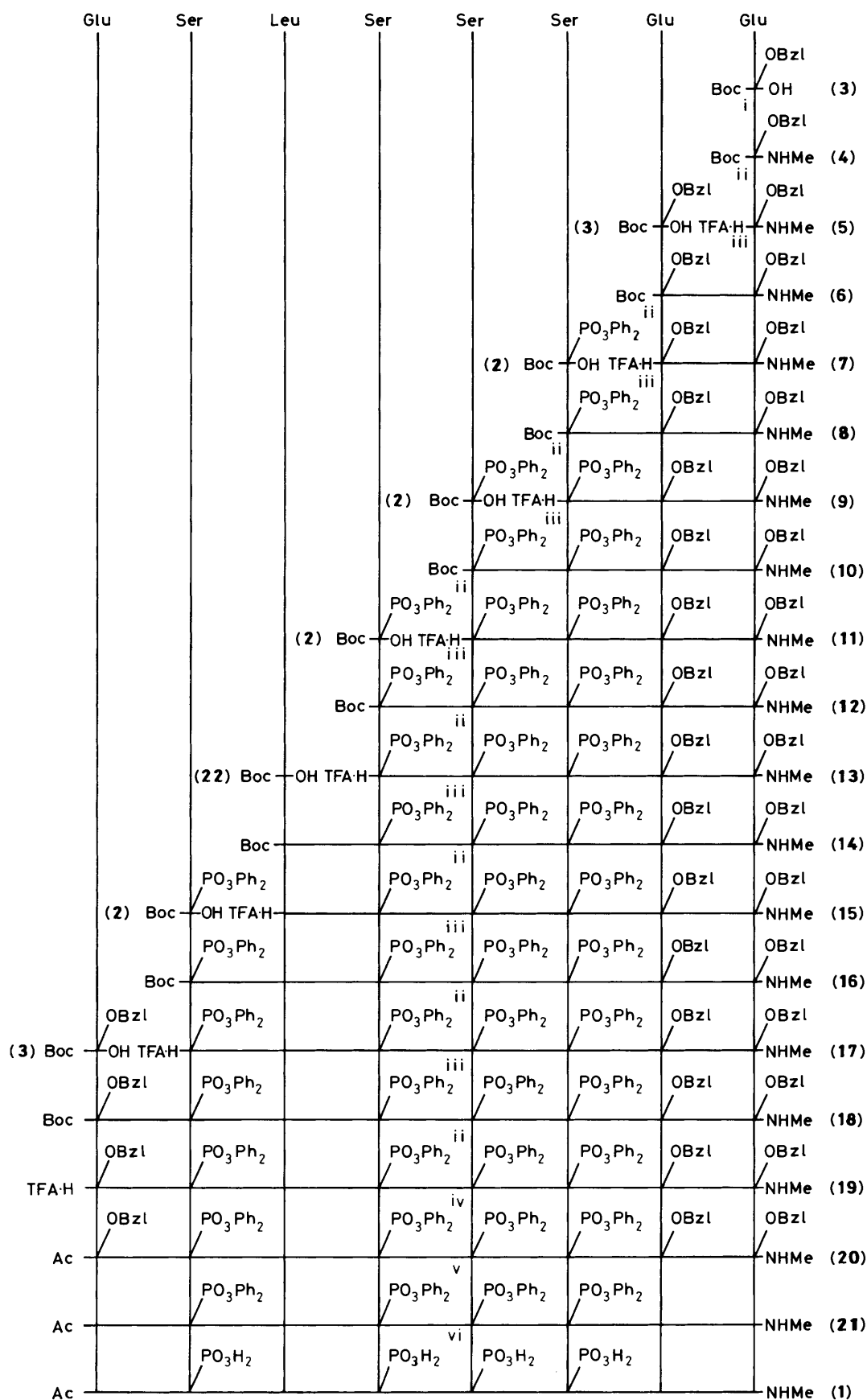
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The title octapeptide was prepared by the synthesis of the fully protected tetra-Ser(PO₃Ph₂)-octapeptide by incorporation of Boc-Ser(PO₃Ph₂)-OH (Boc = *t*-butoxycarbonyl) in conventional Boc/solution phase peptide synthesis, followed by the complete hydrogenolytic cleavage of the phenyl groups from the Ser(PO₃Ph₂)-octapeptide.

Since 1957, the synthesis of simple *O*-phosphoseryl-containing peptides has generally been accomplished by the 'global' phosphorylation of protected serine-containing peptides using diphenyl or dibenzyl phosphorochloridate-pyridine followed by hydrogenolytic removal of the phenyl or benzyl phosphate protecting groups.¹⁻³ However, as we found this synthetic approach unsuitable for the synthesis of large and/or multi-

*P*Ser-containing peptides,⁴ we developed an alternative Ser(PO₃R₂)-peptide synthetic procedure⁵ which featured (a) the incorporation of Boc-Ser(PO₃Ph₂)-OH⁶ (Boc = *t*-butoxycarbonyl) into conventional Boc/peptide synthesis and (b) the use of modified hydrogenation conditions for the complete removal of the phenyl phosphate groups from Ser(PO₃Ph₂)-peptides. While we have already reported the preparation of



Scheme 1. Reagents: i, *N*-methylmorpholine, isobutyl chloroformate, then *N*-methylamine (-20°C , 2 h); ii, 40% TFA- CH_2Cl_2 ; iii, *N*-methylmorpholine, isobutyl chloroformate, (-20°C , 2 h); iv, MeCO_2H , *N*-methylmorpholine, isobutyl chloroformate (-20°C , 2 h); v, 10% Pd/C, 10% AcOH-MeOH; vi, 1.1 equiv. PtO_2 /mmol phenyl group, 50% TFA-AcOH.

the simple *PSer*-tripeptide Glu-*PSer*-Leu⁶ and the multi *PSer*-tripeptide *PSer-PSer-PSer-NHMe*⁷ using this general synthetic procedure, we now report the straightforward synthesis of the complex tetra-*PSer*-octapeptide, Ac-Glu-*PSer*-Leu-*PSer-PSer-PSer-Glu-Glu-NHMe*. This heavily phosphorylated peptide is of particular biochemical interest since this amino acid sequence, which corresponds to regions 14–21 and 5–12 of bovine and human β -casein, respectively, is known to be a prominent calcium-binding region and is thought to be responsible for maintaining the structural integrity of the casein micelle.

The fully protected Ser(PO₃Ph₂)-octapeptide (**20**) was readily prepared in an overall yield of 61% starting with Boc-Glu(OBzl)-NHMe (all couplings proceeding in over 90% yields) by (a) the use of the mixed anhydride coupling procedure for all amino acid condensations, (b) the incorporation of Boc-Ser(PO₃Ph₂)-OH at the required residue positions, (c) the use of 40% trifluoroacetic acid (TFA)-CH₂Cl₂ for cleavage of the Boc group from all the intermediate Boc-peptides, and (d) the use of the isobutoxycarbonyl mixed anhydride of acetic acid for the *N*-acetylation⁸ of the amino terminus of octapeptide (**19**) (see Scheme 1). The incorporation of the four Ser(PO₃Ph₂)-residues into the octapeptide (**20**) was established from its ³¹P n.m.r. spectrum which displayed four distinct phosphorus resonances at δ -11.0, -12.9, -13.0 and -13.2 p.p.m.

The removal of the glutamyl benzyl groups was effected by the hydrogenation of octapeptide (**20**) in 10% AcOH-MeOH with 10% palladium on charcoal to give the Ser(PO₃Ph₂)-octapeptide (**21**) in quantitative yield. Further hydrogenation of this peptide in 50% TFA-AcOH and 1.1 equiv. PtO₂/mmol phenyl group effected the rapid and complete removal of the phenyl phosphate groups, the reaction being complete after 30 min. C₁₈ Reverse-phase h.p.l.c. purification of the crude product (one major, three minor fractions) using an isocratic

elution of 0.1% aq. TFA-9% acetonitrile gave the target tetra-*PSer*-octapeptide (**1**) [fast atom bombardment (f.a.b.) mass spec. (+ve mode) *m/z* 1242 (MH⁺)] in 53% yield.

To our knowledge, peptide (**1**) represents the largest and most complex multi-*PSer*-peptide that has been reported to date. The simple, straightforward, and high-yielding synthesis of (**1**) dictates that the synthetic strategy described above is the method of choice for the general preparation of *PSer*-peptides and is a significant improvement over the traditional 'global' phosphorylation strategy.

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