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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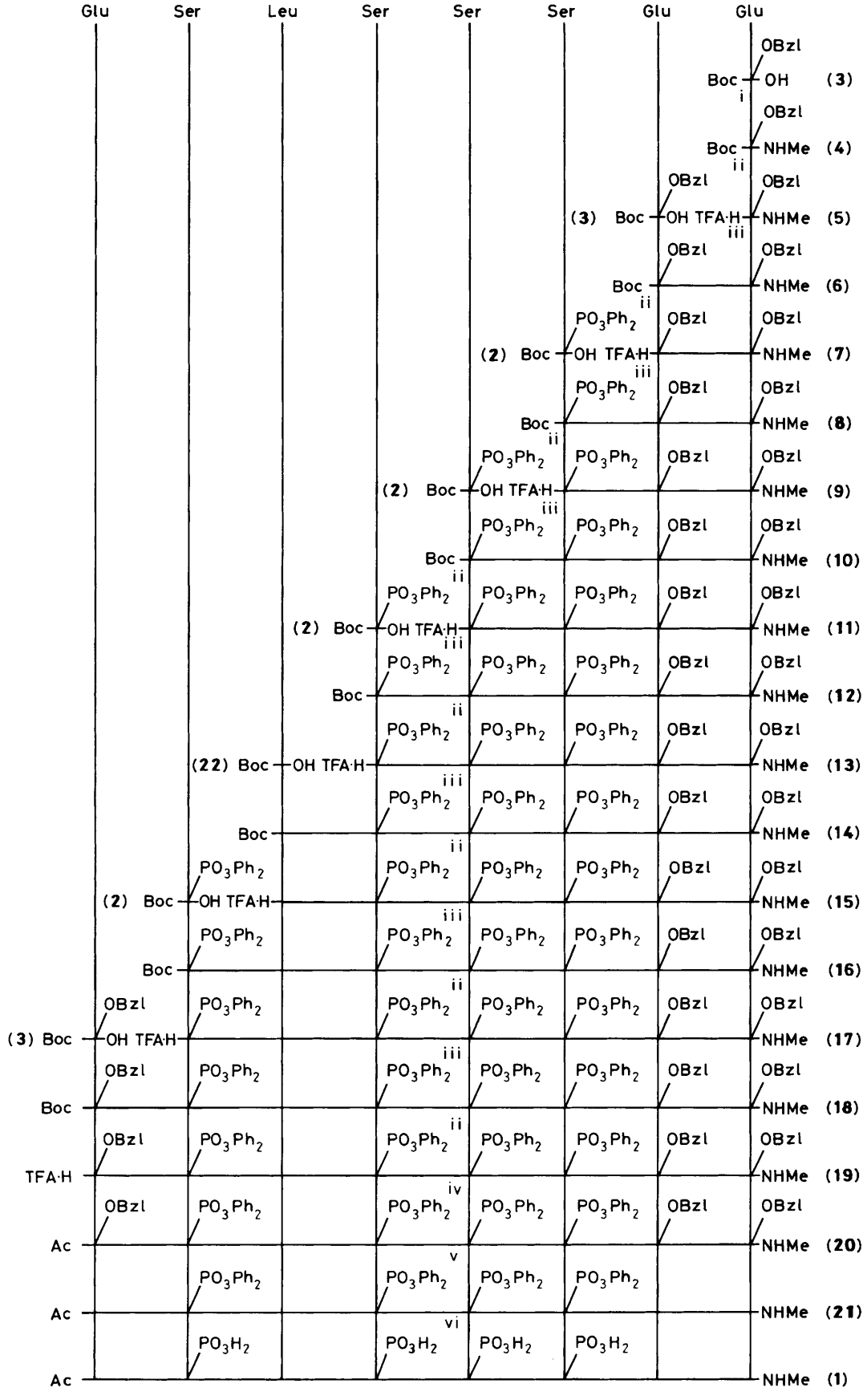
The title octapeptide was prepared by the synthesis of the fully protected tetra-Ser(PO_3Ph_2)-octapeptide by incorporation of Boc-Ser(PO_3Ph_2)-OH (Boc = t-butoxycarbonyl) in conventional Boc/solution phase peptide synthesis, followed by the complete hydrogenolytic cleavage of the phenyl groups from the Ser(PO_3Ph_2)-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(PO_3R_2)-peptide synthetic procedure[5] which featured (a) the incorporation of Boc-Ser(PO_3Ph_2)-OH[6] (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(PO_3Ph_2)-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 *P*Ser-tripeptide Glu-*P*Ser-Leu[6] and the multi *P*Ser-tripeptide *P*Ser-*P*Ser-*P*Ser-NHMe[7] using this general synthetic procedure, we now report the straightforward synthesis of the complex tetra-*P*Ser-octapeptide, Ac-Glu-*P*Ser-Leu-*P*Ser-*P*Ser-*P*Ser-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(PO_3Ph_2)-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(PO_3Ph_2)-OH at the required residue positions, (c) the use of 40% trifluoroacetic acid (TFA)–CH_2Cl_2 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*-acetylation[8] of the amino terminus of octapeptide (**19**) (see Scheme 1). The incorporation of the four Ser(PO_3Ph_2)-residues into the octapeptide (**20**) was established from its ^{31}P 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(PO_3Ph_2)-octapeptide (**21**) in quantitative yield. Further hydrogenation of this peptide in 50% TFA–AcOH and 1.1 equiv. PtO_2/mmol phenyl group effected the rapid and complete removal of the phenyl phosphate groups, the reaction being complete after 30 min. C_{18} 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-*P*Ser-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-*P*Ser-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 *P*Ser-peptides and is a significant improvement over the traditional 'global' phosphorylation strategy.

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