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THE EFFICIENT SYNTHESIS OF A O-PHOSPHOTYROSINE-CONTAINING PEPTIDE USING MODERN DEPROTECTION METHODS

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Abstract: The O-phosphotyrosine-containing peptide Pro-pTyr-Val was prepared in high yield by the incorporation of Boc-Tyr(PO_3Me_2)-OH in peptide synthesis and the use of TFMSA/TFA/DMS/ m-cresol or 1M TMSBr/TFA/m-cresol for the final peptide deprotection.

The recent recognition that tyrosine specific kinases are involved in cellular transformations[1] has created the need for synthetic O-phosphotyrosine-containing peptides for use as model substrates. However, two recently reported[2,3] syntheses of small O-phosphotyrosine-containing peptides using Boc-Tyr(PO_3R_2)-OH (R= Me, Bzl) in Boc/solid-phase peptide synthesis have highlighted the need for more efficient deprotection steps; 45% HBr/AcOH giving poor yields[2] (and also acetylation of β-hydroxyamino acids), 10% TMSBr/CH_3CN having low peptide dissolution strength[4] and HF giving very low yields and extensive side-product formation.[3] In this letter, we examine the stability of the Tyr(PO_3Me_2)-residue under HF conditions and report on the application of two recently reported treatments for the efficient deprotection of a Tyr(PO_3Me_2)-containing tripeptide.

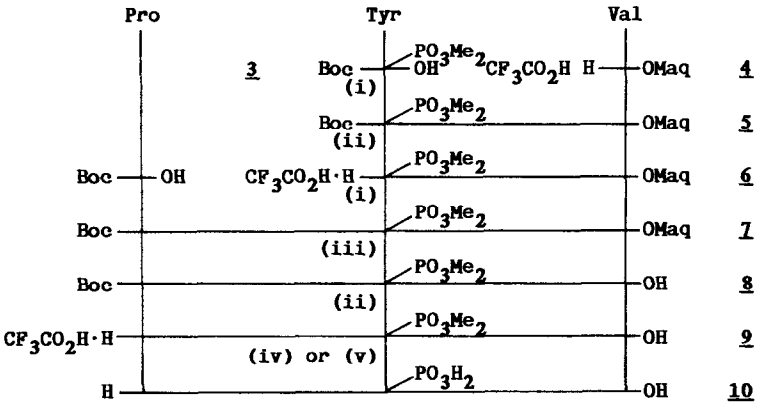
To evaluate the use of HF deprotection, the model substrate HCl.H-Tyr(PO_3Me_2)-OH **1** was subjected to both 'high' HF and 'low' HF[5] treatments. Quantitative amino acid analysis of the 'high' and 'low' HF-treated peptide residues established that 15% and 90% dephosphorylation had occurred respectively. These results show that firstly, HF does not effect efficient methyl dealkylation and secondly, DMS may facilitate dephosphorylation under 'low' HF conditions. The high rate of phosphate cleavage in (ii) suggests that the dimethyl phosphate group may be a suitable protecting group for tyrosine in peptide synthesis.

$$\underset{\mathbf{1}}{\text{HCl.H-Tyr}(PO_3Me_2)\text{-OH}} \xrightarrow[\text{(ii) HF/DMS/m-cresol (25:65:10), 2h, 0°C}]{\text{(i) HF/anisole (9:1), 45 min, 0°C or}} \underset{\mathbf{2}}{\text{H-Tyr-OH}} \quad \begin{matrix}15\%\\90\%\end{matrix}$$

In order to overcome the synthetic limitations in the use of 45% HBr/AcOH and 10% TMSBr/CH_3CN for phosphate deprotection, the recently reported TFMSA/TFA/DMS/m-cresol[6] and 1M TMSBr/TFA/m-cresol[7] treatments were examined for potential use in effecting the efficient demethylation of H-Pro-Tyr(PO_3Me_2)-Val-OH **9**. The synthesis of tripeptide[8] **9** was accomplished by the incorporation of Boc-Tyr(PO_3Me_2)-OH **3**[2,3] in conventional Boc peptide synthesis using the excess mixed anhydride coupling procedure followed by sodium dithionite reduction of the 2-methylanthraquinone (Maq)[9] group and acidolytic cleavage of the Boc group (see Scheme 1).

The low temperature treatment of tripeptide **9** (52 mg) with TFMSA/TFA/DMS/m-cresol

Scheme 1



(i) NMM/IBCF, (ii) 40% TFA/CH_2Cl_2, (iii) $Na_2S_2O_4$/1M $NaHCO_3$, (iv) TFMSA/TFA/DMS/m-cresol (v) 1M TMSBr/TFA/m-cresol

(10:50:30:10 v/v, 3ml, 4h, 0°C) was monitored using ^{31}P NMR spectroscopy and indicated complete demethylation occurred after 4 hours. Subsequent semi-preparative C_{18} RP-HPLC purification of the crude residue gave the PTyr-tripeptide 10 in 66% yield, its structure being confirmed by its ^{13}C NMR[10] and ^{31}P NMR spectra [$\delta(H_2O)$: -3.6]. Alternatively, demethylation of peptide 9 (37 mg) using 1M TMSBr/TFA/m-cresol (3 ml, 10h, 0°C) also proceeded smoothly and afforded tripeptide 10 in 75% yield, its structure being confirmed by its ^{13}C NMR spectra.[10] In contrast to the use of 10% TMSBr/CH_3CN, the high dissolution strength and high reactivity of 1M TMSBr/TFA indicates that this procedure provides a suitable and efficient deprotection method for Tyr(PO_3Me_2)-containing peptides.

This work demonstrates that HF is unsuitable for the deprotection of Tyr(PO_3Me_2)-residues and that both TFMSA/TFA/DMS/m-cresol and 1M TMSBr/TFA/m-cresol are suitable for the efficient deprotection of Tyr(PO_3Me_2)-containing peptides. The use of these latter procedures in the synthesis of larger PTyr-containing peptides is currently in progress.

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8. Tripeptide 9 was chosen so as to permit specific monitoring of the dimethyl phosphate group without the added complication of the amino and carboxyl protecting groups.
9. The Maq group {Kemp, D. S. and Reczek, J., Tetrahedron Lett., 1031, (1977)} was selected for ester protection so as to obtain solid products. In past experiences, we have found that the use of the benzyl group gives oil products which are difficult to purify.
10. ^{13}C NMR $\delta(D_2O)$ 10: 174.4, 172.3, 169.1, 150.9 (d, 7.32 Hz), 131.7, 130.3, 120.7 (d, 3.66 Hz), 59.4, 58.5, 55.5, 46.5, 36.3, 30.0, 29.7, 23.6, 18.1, 17.3.

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