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A Rapid Dealkylation of Phosphonate Diester for the Preparation of 4-Phosphonomethylphenylalanine-Containing Peptides

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Abstract: Peptides containing O,O-diethylphosphonomethylphenylalanine [Pmp(OEt)$_2$] were synthesized by standard solid phase protocols. Cleavage and deprotection were achieved rapidly at room temperature via a 2-step process. An initial trifluoroacetic acid (TFA) -mediated deprotection and cleavage reaction, followed by a rapid trimethylsilyl-iodide (TMS-I)/acetonitrile (MeCN) cleavage of the phosphonate diester provided the product in practical quantities. The use of MeCN appears to be crucial for the rapid removal of the alkyl groups.

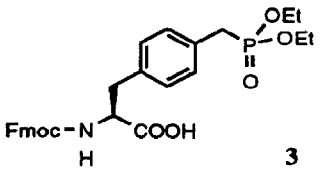
Protein phosphorylation on tyrosine provides a major means of cellular signal transduction.[1] Specific sequences spanning certain phosphotyrosine residues form key recognition motifs for protein-protein association mediated by src-homology 2 (SH2) domains of cytosolic proteins.[2] These interactions play crucial roles in protein tyrosine kinase cellular signalling pathways.

We are interested in using a non-hydrolyzable analogue of phosphotyrosine to evaluate the interaction between a 19 amino-acid peptide (**1**) derived from the intracellular portion of the IgE receptor γ-chains and the two SH2 domains of the intracellular protein tyrosine kinase, Syk.[3] However, preparation of phosphonic acid containing compounds is hampered by difficult ester hydrolysis, which requires vigorous conditions often over extended periods of time leading to degradation of the desired product.[4-9] We report herein a rapid preparation of the phosphonomethylphenylalanine (Pmp) containing peptide analogue (**2**) without recourse to extended and harsh hydrolysis conditions.

Ac-Asp.Gly.Val.**Xaa**.Thr.Gly.Leu.Ser.Thr.Arg.Asn.Gln.Glu.Thr.**Xaa**.Glu.Thr.Leu.Lys-NH_2

1: Xaa = Tyr; **2**: Xaa = Pmp

The synthesis of the desired peptide was expedited by the use of the commercially available amino acid derivative, Fmoc-O,O-diethyl-phosphonomethylphenylalanine [Fmoc-L-Pmp(OEt)$_2$OH, **3**].[10] The 19 amino acid peptide was assembled on an ABI 433A peptide synthesizer using standard protocols. Details of the synthesis are shown in Scheme 1. Fmoc-L-Pmp(OEt)$_2$OH was manually delivered into the reaction vessel due to high



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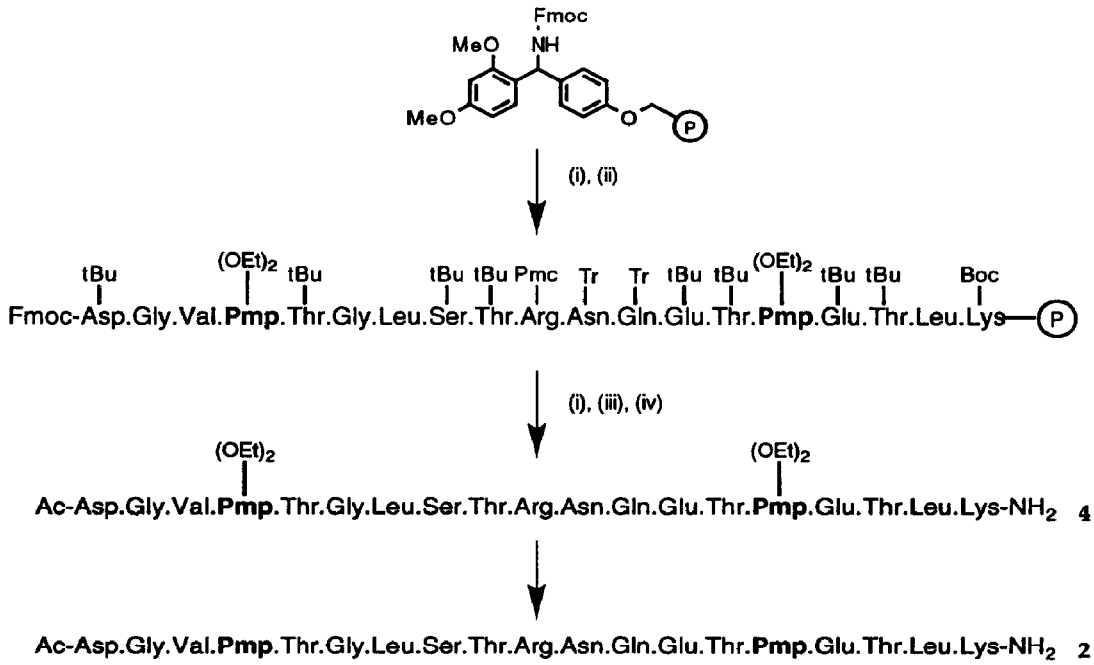
viscosity of the activated amino acid. At the conclusion of the solid phase synthesis, all acid labile protecting groups were removed with simultaneous detachment of the peptide from the solid support (*ca.* 1.5 g) by means of 95% aq.TFA, 1,2-ethanedithiol, anisole, phenol; 40:2:2:0.5 under an atmosphere of nitrogen gas at room temperature. Figure 1A shows the HPLC profile of the crude product (**4**). Mass spectrometric analysis of a purified portion indicated tetraethyl protected diphosphono peptide. Treatment of samples of **4** (10 mg each) with trifluoromethane-sulphonic acid/TFA/dimethylsulphide/m-cresol, trimethylsilylbromide (TMSBr), TMSBr/ dichloromethane (DCM); TMSBr/dimethylacetamide (DMA); TMSBr/MeCN, all gave a mixture of starting material and various levels of ethyl protection. Trimethylsilyliodide (TMSI)/-40 °C; TMSI/0 °C to RT; TMSI/DCM; TMSI/DMA, all gave starting material and byproducts which do not have the measured mass of the expected product.



(i) Deprotection: 20% Piperidine in DMA, (ii) Coupling: Fmoc-amino acid/HBTU/HOBt/DIEA, (iii) Ac_2O/Pyridine, (iv) 95% aq.TFA/EDT/anisole/phenol.

Scheme1

However, on suspending the crude peptide (20 mg), in MeCN (1 mL), followed by dropwise addition of TMSI (1 mL) over 10min at room temperature, a clear pale yellow solution resulted.[11] Complete formation of desired product, **2**, was indicated by HPLC within 35 min. HPLC purification of this material followed by mass spectrometric analysis confirmed the desired molecular weight (Figure 1B).

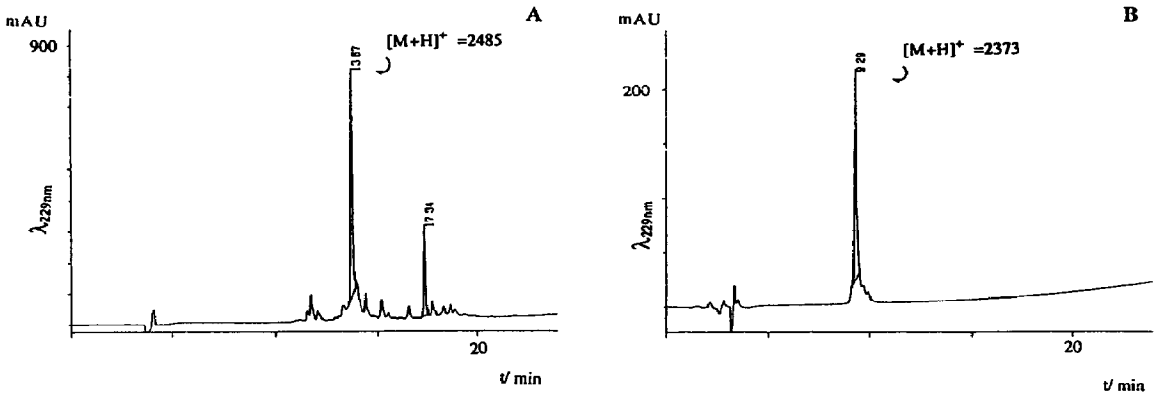


Figure.1 A: HPLC[12] of crude sample **4**; B: HPLC of purified **2**.

Thus, in a typical experiment, TMSI (2 mL) was added dropwise to a suspension of **4** (110 mg, ca. 75% purity) in MeCN (2 mL) at RT and reaction monitored by HPLC. After 100 min complete reaction was achieved. Reaction was terminated by removal of MeCN *in vacuo* followed by hydrolysis of the TMS ester intermediates with cold 10% HOAc/H_2O (25 mL). Extraction of the aqueous solution with diethylether gave a clear colourless solution. Lyophilization of the solution revealed a pale yellow powder (~95 mg) which was purified by HPLC to give 35 mg (50% yield) of homogenous product **2**. The product gave the expected mass spectral and amino acid analyses. Biological evaluation of this peptide and others in this series will be reported elsewhere.

Acknowledgements:

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10. NEOSYSTEM Laboratoire, 67100 Strasbourg, France; Chiral synthesis of Pmp derivative is achievable according to reference 8.
11. TMSCl/NaI/MeCN at reflux has previously been used in the cleavage of phosphonate diethyl esters: Rachon, J. *Synthesis* **1984**, 219-222.
12. Analytical HPLC were run on Hewlett Packard 1050 on Kromasil C8 column (250 x 4.6 mm), 5μm, 100Å. Elution conditions: 15-100% B [A= 0.1% aqueousTFA; B=60/40 MeCN/H_2O (0.1% TFA)] over 40min. Semi-preparative HPLC was carried out on a Kromasil C8 column (250 x 20 mm), 10μm, 100Å, eluting with 25-47% B over 20min.

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