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Solid Phase Synthesis of Phosphonopeptides from Fmoc Phosphonodipeptides

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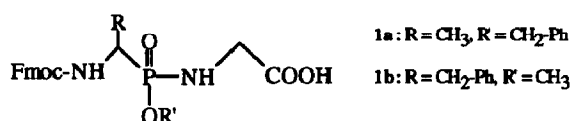
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Key Words : Phosphonopeptide ; Solid phase ; Fmoc strategy ; Phosphonodipeptide precursors ; allylic protection

Abstract : The solid phase synthesis of two phosphonopeptides was carried out from a N-Fmoc protected dipeptide precursor containing the P-N bond and a benzyl or allyl carboxylic protection easily removable under neutral conditions.

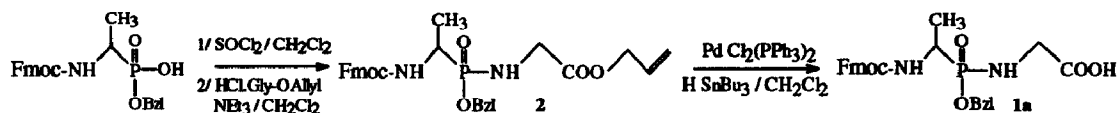
Phosphonopeptides have biological interest as protease inhibitors¹ or haptens for catalytic antibodies synthesis.² The Fmoc strategy developed in our laboratory allowed us the synthesis of various benzyl ester protected phosphonopeptides,³ but the limitations of this method for longer pseudopeptides prompted us to develop the solid phase procedure.

Our previous experiments have shown that the P-N bond formation cannot be formed directly. The solid phase phosphonopeptide synthesis is possible by incorporation of a preformed phosphonodipeptide **1** containing the P-N bond and the O-benzyl (**1a**) or O-methyl (**1b**) protection.



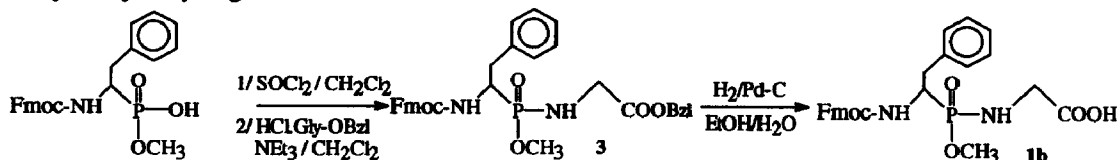
The access to the synthon **1** requires a carboxylic protection removable under neutral conditions compatible with the Fmoc and either the O-Me or O-Bzl protection, and the acidic lability of the P-N bond.

The allyl carboxylic group protection, removable under neutral conditions with no use of catalytic hydrogenation,⁴ is well-adapted to the obtention of **1a**.

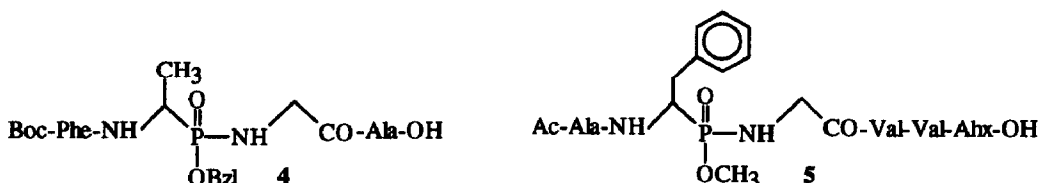


The phosphonodipeptide **2** was obtained from the mono benzyl ester of the Fmoc α -aminophosphonic acid.^{3, 5} The allylic protection was removed in CH_2Cl_2 with 2 equivalents of tributyltin hydride in presence of $\text{PdCl}_2(\text{PPh}_3)_2$ under an inert atmosphere to give the corresponding tributyltin ester,⁴ which was converted into the free carboxylic acid **1a** upon protolysis.⁶

The dipeptide **1b** was obtained by using the benzyl carboxylic ester protection which was further easily removed by catalytic hydrogenation.⁷



The incorporation of the Fmoc phosphonodipeptide **1a** or **1b** with a free C-terminal moiety on solid support has been quite promising. For example, we have been able to prepare the phosphonopeptides **4** and **5**⁸: the dipeptides **1a** or **1b** were coupled by their carboxy terminus to the N-terminal peptidyl resin using BOP/DIEA as reagents.⁹ The elongation was performed by Fmoc deprotection with piperidine followed by acylation with BOP/DIEA.



This Fmoc solid phase strategy can be applied to the synthesis of pseudopeptides with various phosphorus protection and position of the phosphoramidate bond in the peptide chain.

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- Compounds **1a** and **1b** were obtained in 58 % and 44 % yields, respectively after purification. Compounds were characterized by ³¹P NMR and ¹H NMR.
- The crude dichloromethane mixture **4** containing the tributyltin ester was evaporated and redissolved in ethyl acetate. The mixture was washed with water at pH 2-3 by addition of HCl 10 %. In IR absorption, the absence of any residual peak at 1650 cm⁻¹ (CO₂SnBu₃) indicated a complete protolysis. The organic layer was dried and the solvent evaporated under reduced pressure. The brown oil obtained was dissolved in acetonitrile and washed 5-6 times with hexane to eliminate the by-products of the reaction (bis(tributyltin)oxide, hexabutylstannane, tributyltin chloride...). The acetonitrile was evaporated. In a last treatment, the carboxylic acid dissolved in water containing 2 eq. of CO₃Na₂ was washed with ether to eliminate the catalyst derivatives and lyophilised. Compound **1a** was obtained in 75 % yield and was characterized by MS, ¹H NMR and ³¹P NMR.
- Compound **1b** was obtained quantitatively from its benzyl ester after hydrogenation in ethanol/water 50/50 with a catalytic amount of Pd-C.
- The pseudopeptide **5** is used as a transition state analog in the HIV aspartyl protease substrate hydrolysis for the production of potential catalytic antibodies (work is in progress).
- In a typical procedure, 3 eq of **1b** were coupled to Val-Val-Ahx-Expansin^R (Ahx is 6-aminohexanoic acid) in presence of 3 eq. BOP and 5 eq. DIEA. Coupling reaction time was determined by Kaiser test. Fmoc group was cleaved by piperidine. 2.5 eq. Ac-Ala was coupled with BOP and DIEA. Final cleavage was performed by use of 2 eq. NaOH 1N in isopropanol/water 70/30. The structures of **4** and **5** were determined by MS, ³¹P NMR, ¹H NMR, 2D NMR (COSY).

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