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Solid Phase Synthesis of Phosphinic Peptides

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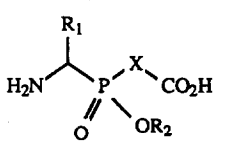
Key words: Phosphinic peptides; SPPS; BOP; PyBOP.

***Abstract**: The absence of a coupling reaction between a phosphinic acid and an amino ester during activation with the reagents BOP or PyBOP allowed the synthesis of phosphinopeptides from phospho-analogues of dipeptides, unprotected on the phosphinic acid.*

Phosphopeptides have been described as protease inhibitors[1] and haptens for the preparation of abzymes possessing esterase activity[2]. The phosphorus moiety, i.e. phosphonamidic, phosphonic or phosphinic, mimics the transition state of the hydrolysis.

Peptide elongation involves formation of amide bonds at the C-terminus and N-terminus for type **1** compounds. Coupling reactions in solution have been successfully performed using a mixed anhydride, DCC, DCC/HOBt[1a-d] or BOP[3] on type **1a** or **1b** compounds possessing an ester-protected phosphorus acidic function, which requires subsequent deprotection of the phosphorus acid. Coupling in solution with type **1c** compounds (unprotected phosphinic acid) leads to high yields of the desired pseudopeptide when using carbonyldiimidazole[4], but only moderate to low yields when using DCC/HOBt even with very long reaction times[1e]. Moreover, DPPA activation can produce rearrangement reactions[5].



1a : X = $(CH_2)_n$, CH_2-CH(R) , CH(R) ; R_2 = alkyl

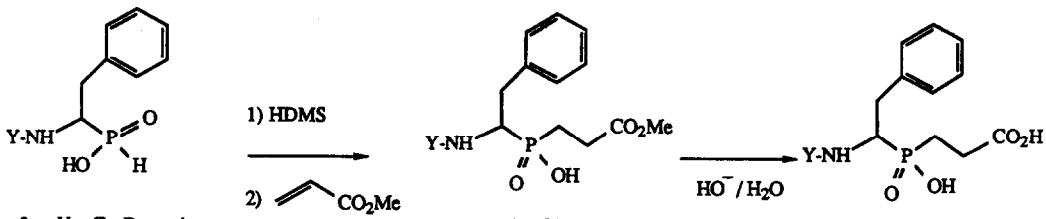
1b : X = OCH_2 , OCH(R) ; R_2 = alkyl

1c : X = $(CH_2)_n$, CH_2-CH(R) , OCH(R) ; R_2 = H

Coupling with type **1c** phosphinic acids is clearly of interest. This is a means to avoid protection and deprotection steps and simplify analysis of the pseudopeptides formed, since ester-protection introduces a chiral centre on the phosphorus atom, thus increasing the number of diastereoisomers.

In the present paper, we describe the conditions for the synthesis of pseudopeptides using the phosphinic moiety **1c** (R_1 = CH_2Ph, X = $(CH_2)_2$, R_2 =H) with BOP[6] or PyBOP®[7] as coupling reagents, and their application to the first phosphinopeptide solid phase synthesis.

Compounds **2a** and **2b**[8] were obtained from the corresponding amino-phosphonous acid[9] by reaction with Z-Cl or Boc_2O. Optically pure compound **2c**[10] was obtained in the same way following resolution of the amino-phosphonous acid, as described by Baylis[9]. Successive reaction with hexamethyldisilazane (HMDS) and methyl acrylate, as described by Boyd[11], allowed formation of compounds **3**, which were then saponified into diacids **4**.

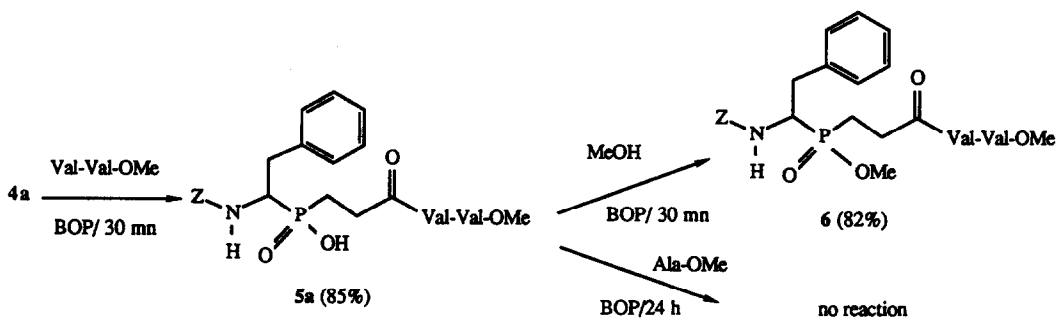


2a : Y = Z ; Racemic — **3a** (92%) — **4a**

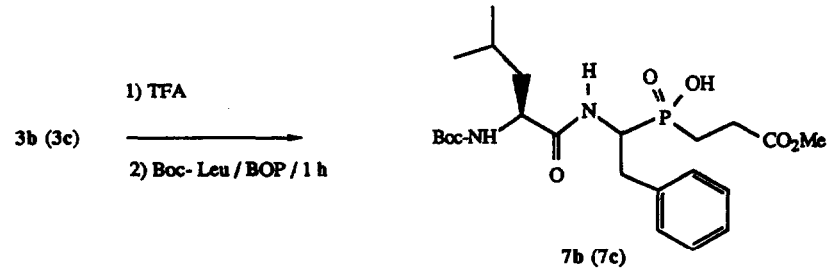
2b : Y = Boc ; Racemic — **3b** (95%) — **4b**

2c : Y = Boc ; Optically pure — **3c** (52%) — **4c**

Coupling of diacid **4a** in solution (DMF) with Val-Val-OMe using BOP/DIEA gave compound **5a** and no phosphinamide bond was formed despite the use of two equivalents of coupling reagent. The difficulty in forming a P-N linkage was confirmed by the fact that compound **5a** did not react with Ala-OMe in the presence of BOP/DIEA although the phosphinic acid was activated since the same compound gave phosphinate **6** by reaction with BOP/DIEA and methanol[12]. These results are in accordance with the reactivity of mixed carboxylic-phosphinic anhydrides which react with amines solely at the carbonyl group,[13] and with the low yields obtained when coupling phosphinic acids and amino-esters using BOP.[14]

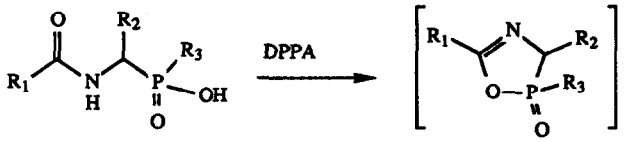


We were thus able to chemoselectively obtain amide bonds at the C-terminus in type **1c** compounds. Peptide elongation can also be performed at the N-terminus, without formation of a P-N linkage, and compounds **3b** (or **3c**), after TFA-deprotection and BOP-mediated coupling with Boc-Leu, gave pseudopeptides **7b** (or **7c**)[15].



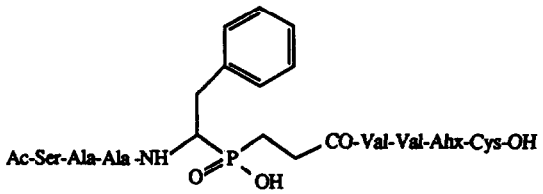
7b (7c)

To elongate type 7 compounds on the N-terminal side, we studied the behaviour of this compound following BOP-activation since Bartlett[5] has observed rearrangement during activation of type 8 compounds with diphenylphosphoryl azide (DPPA). The first step of this activation involves phosphaoxazolone 9. In reference to the main epimerization mechanism of peptide synthesis involving oxazolone formation, passing through the phosphaoxazolone could also lead to epimerization of the α-carbon. We observed that the diastereoisomerically pure compound 7c[15] was unchanged after 48h BOP-activation, proving that no rearrangement or epimerization occurs with this coupling reagent.



8 9

Taking into account these results we used solid phase to synthesize some phosphinic peptides. From 3b or 3c, we obtained peptides 10a (diastereoisomeric mixture) or 10b (pure compound) by SPPS on Merrifield resin, according to a standard Boc/TFA technique with PyBOP® as coupling reagent [16].



10a (10b: diastereoisomerically pure)

This demonstrates that peptide synthesis can be achieved with a phosphorus-unprotected phosphinic acid through linkage at the C- and N-terminal ends, without formation of P-N linked compounds or epimerization. We are presently using this method for the synthesis of other phosphinic and phosphonic peptides.

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REFERENCES AND NOTES

1. (a) Dreyer, G.B.; Metcalf, B.W.; Tomaszsek Jr, T.A.; Carr, T.J.; Chandler III, A.C.; Hyland, L.; Fakhoury, S.A.; Magaard, V.M.; Moore, M.L.; Strickler; J.E.; Debouck, C.; Meek, T.D. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9752-9756.
   (b) Bartlett, P.A.; Hanson, J.E.; Gianoussis, P.P. *J. Org. Chem.* **1990**, *55*, 6268-6274.
   (c) Morgan, B.P.; Scholtz, J.M.; Ballinger, M.D.; Zipkin, I.D.; Bartlett, P.A. *J. Am. Chem. Soc.* **1991**, *113*, 297-307.
   (d) Bartlett, P.A.; Kezer, W.B. *J. Am. Chem. Soc.* **1984**, *106*, 4282-4283.
   (e) Allen, M.C.; Fuhrer, W.; Tuck, B.; Wade, R.; Wood, J.M. *J. Med. Chem.* **1989**, *32*, 1652-1661.
2. Pollack, S.J.; Hsiun, P.; Schultz, P.G. *J. Am. Chem. Soc.* **1989**, *111*, 5961-5962.
3. Coutrot, Ph.; Grison, C.; Charbonnier-Gérardin, C. *Tetrahedron* **1992**, *48*, 9841-9868.
4. Grobelny, D.; Goli, U.B.; Galardy, R.E. *Biochemistry* **1989**, *28*, 4948-4951, see supplementary material.
5. Bartlett, P.A.; Acher, F. *Bull. Soc. Chim. Fr.* **1986**, *5*, 771-775.
6. Castro, B.; Dormoy, J.-R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219-1222.
7. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205-208.
8. All compounds obtained were characterized by elemental analysis, MS, ^{31}P and ^{1}H NMR.
9. Baylis, E.K.; Campbell, C.D.; Dingwall, J.G. *J. Chem. Soc., Perkin. Trans. 1* **1984**, 2845-2853.
10. The absolute configuration of this compound was not determined.
11. Boyd, E. A.; Regan, A.C.; James, K. *Tetrahedron Lett.* **1992**, *33*, 813-816.
12. We are presently using this reaction to synthesize phosphonates using BOP or PyBOP®.
13. Ramage, R.; Hopton, D.; Parrot, M.J.; Richardson, R.S.; Kenner, G.W.; Moore, G.A. *J. Chem. Soc., Perkin. Trans. 1* **1985**, 461-470.
14. Elhaddadi, M.; Jacquier, R.; Petrus, C.; Petrus, F. *Phosphorus Sulfur and Silicon* **1991**, *63*, 255-259.
15. **7b** was obtained with 72% yield after HPLC purification. In ^{31}P NMR, **7b** gave two signals at $\delta = 45.5$ and 44.7 ppm and two HPLC peaks at 8.7 and 9.4 min (Ultrabase C_8 SFCC column, 4.5 x 150 mm, CH_3CN-H_2O- 0.1% TFA, 1.5 ml/min, gradient (30 to 80% CH_3CN in 20 min)). **7c** : ^{31}P NMR, δ = 44.5 ppm; HPLC peak at 9.4 min.
16. **10a** (Ahx is 6-aminohexanoic acid) was synthesized from 3g Boc-Cys(Meb)-resin (0.44 mmol/g) in dichloromethane using 2.5 equivalents Boc-amino acid and PyBOP, in the presence of DIEA, according to the usual method[7]. Coupling reaction times (20 to 60 min) were determined by the Kaiser test. The Boc group was cleaved by TFA. For solubility purposes, coupling of **4b** was performed in DMF. For the coupling of **4b**, Ala, Ala and Ser(OBzl), 2.5 equivalents of Boc-AA and 5 equivalents of PyBOP were used. Final cleavage was performed with HF. Peptide yield was 55% after HPLC purification (C_8, Ultrabase, 10 µ, 25 x 500 mm, SFCC). **10b** was obtained in a similar way. The structures were determined by elemental analysis, amino-acid analysis, ^{31}P and ^{1}H NMR and 2D ^{1}H NMR (COSY and NOESY). Purity of peptide **10b** was assessed by ^{31}P and ^{1}H NMR by comparison with diastereoisomeric mixture **10a**, epimerization was $\leq 0.5\%$ within the limits of the detection method.

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