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Solid Phase Synthesis of Phosphonopeptides

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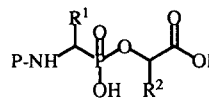
Abstract: A solid phase synthesis of a phosphonopeptide is described, in which a type **1** aminophosphonate with a free phosphonic acid was coupled just like an amino acid, using BOP as reagent.

Phosphonopeptides containing a transition state analog of the hydrolysis of the scissile amide bond are of great interest in the development of enzyme inhibitors[1] and haptens for the production of catalytic antibodies possessing esterase activity.[2]

Solution synthesis of such peptides is well documented.[1b,c] The use of solid phase synthesis, which has several advantages has been described in only one recent report.[3] Campbell coupled a *N*-protected aminophosphonate monoester to a hydroxypeptidyl-resin by a Mitsunobu reaction to prepare a phosphonate mixed diester peptidyl-resin, with an inversion of the configuration at the hydroxy-bearing atom. The phosphonopeptide was elongated at the *N*-terminus. The free phosphonopeptide was finally obtained by selective hydrolysis of the phosphonate protection followed by cleavage from the resin.

We present here a different methodology for the solid phase synthesis of phosphonopeptides, using type **1** aminophosphonates with an unprotected phosphonic acid.



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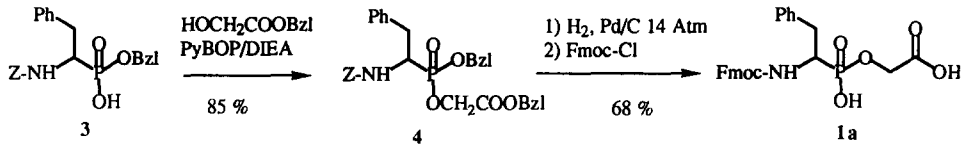
Given the fact that BOP or PyBOP-activated phosphinic acids do not react with amines, we used aminophosphinates with an unprotected phosphinic acid group for solid phase synthesis of phosphinopeptides.[4]

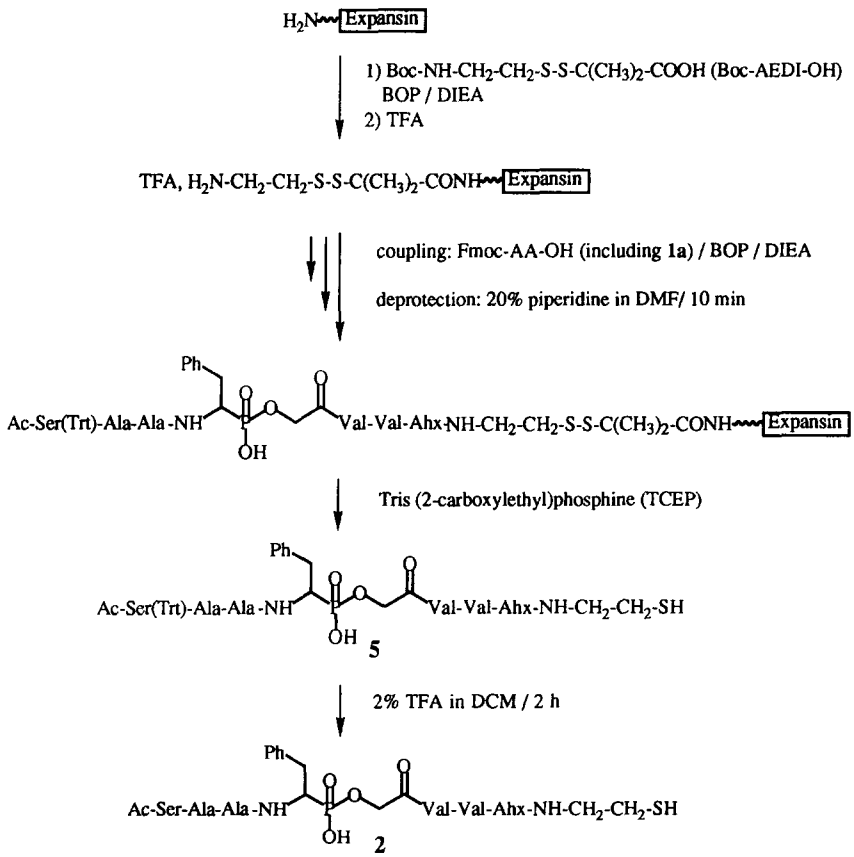
With phosphonic acids, the situation is more complicated. The BOP or PyBOP-promoted reaction of phosphonic acid monoesters gives benzotriazolyl esters (Bt esters) which react with alcohols to produce mixed diesters in high yields.[5,6] With amines, Dumy[7] reported the formation of a phosphonamidate bond between monoesters of phtalylglycylphosphonic acid and various α-amino esters (yields: 60-65%). However, when the amino phosphonate was Z(or Fmoc)-protected, the phosphonamidate bond was not formed, using either BOP or PyBOP.[8,9,10] Moreover, we showed[6] that, in the presence of BOP, monomethyl phenylphosphonate reacts with H-Ala-OMe to give the corresponding phosphonamidate, albeit with moderate yield (64%) despite the use of an excess of reagents and a long reaction time.

Thus, Bt esters of phosphonic acid monoester formed during BOP (PyBOP)-promoted reactions are very reactive with alcohols but poorly reactive with amines. Moreover, these Bt esters are highly reactive with water.[6] Therefore, since SPPS is conducted under nonanhydrous conditions, we anticipated that no P-N bond would be formed despite the use of a free phosphonic acid. Consequently, we used aminophosphonate **1a** with an unprotected phosphorus moiety for solid phase synthesis of phosphonopeptide **2**.



Aminophosphonate **1a** was obtained from the phosphonate monoester **3**. The mixed diester **4**, synthesized using a procedure described previously,[5,6] was completely deprotected by hydrogenolysis and then allowed to react with Fmoc-Cl to give the Fmoc-amino phosphonate **1a** in 68% yield.

Scheme 1. Principle of the Solid Phase Synthesis of **2**



To synthesize phosphonopeptide **2**, we followed a methodology developed by Méry and Brugidou.[11,12] The use of an Expansin resin modified by an AEDI disulfide handle allowed the release of the peptide with a

thiol group at the C-terminus (see Scheme 1). This thiol moiety should permit the direct coupling of the phosphonopeptide to form a conjugate for use in antibody production.[13]

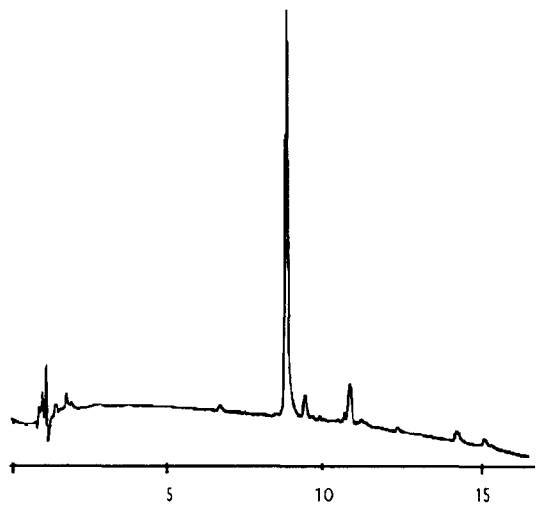
Phosphonopeptide **2** was synthesized from 1 g of H_2N-Expansin (0.53 mmol/g). The disulfide handle *N*-Boc aminoethyldithio-2-isobutyric acid (Boc-AEDI-OH) was introduced using BOP. The Boc protection was removed by TFA. The coupling conditions of the successive Fmoc-amino acids (including **1a**) are summarized in Table 1. After introduction of **1a**, the quantity of BOP/DIEA was increased because of the consumption of BOP by the free phosphonic acid. The peptide was cleaved from the resin by treatment with tris (2-carboxyethyl)phosphine (TCEP) and gave crude peptide **5** whose chromatographic profile shown in Scheme 2 proved the efficiency of the method. Crude peptide **5** was treated with acid (2% TFA in DCM in the presence of TFE and EDT) to remove the trityl group on the serine residue. At this stage, diastereomers **2a** and **2b** were separated (overall yield: 32%) by preparative HPLC (SFCC Ultrabase C8, 10 µm, 25x50 mm). The structures were assessed by ^{31}P and ^{1}H NMR, MS, HR-MS and 2D ^{1}H NMR (COSY and TOCSY).

Table 1. Conditions for the Solid Phase Synthesis of Phosphonopeptide **2**

Amino acid	Equiv of amino acid	Equiv of BOP ; DIEA	Coupling time (min)
Boc-AEDI	1.2	2 ; 4	15
Fmoc-Ahx[1]	2	2 ; 5	15
Fmoc-Val	2	2 ; 5	15
Fmoc-Val	2	2 ; 5	45
1a	2	4 ; 7	30
Fmoc-Ala	2	4 ; 7	15
Fmoc-Ala	2	4 ; 7	15
Fmoc-Ser(Trt)	2	4 ; 7	15
Ac	Ac_2O / DIEA		10

[1] Ahx: 6-aminohexanoic acid

Scheme 2. Chromatographic Profile[1] of Crude Peptide **5**



[1] HPLC conditions: SFCC ultrabase C8 5 µm, 4.6x150 mm; gradient CH_3CN-H_2O: 30-70 to 100-0 in 20 min; 1.5 mL/min; λ 214 nm

In order to show that the synthesis proceeded with no epimerization, we also synthesized the diastereomeric pure phosphonopetide **2a** from homochiral *(R)*-**1a**.[14] HPLC comparison of the crude peptide with diastereoisomeric mixture **2** revealed that epimer **2b** was absent.

In conclusion, using the BOP reagent, solid phase synthesis of phosphonopeptides can be achieved from aminophosphonates **1** unprotected on the phosphorus moiety, with high efficiency and with neither formation of a P-N compound nor epimerization. Using this methodology, the solid phase synthesis is simplified and the final selective hydrolysis of the mixed phosphonate is avoided.

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