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Shortcut to Fmoc-Protected Phosphinic Pseudodipeptidic Blocks

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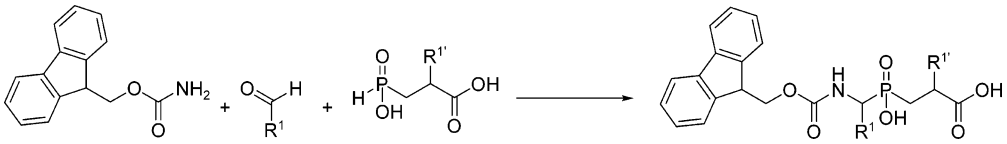
ABSTRACT



A three-component condensation reaction of Fmoc-carbamate, aldehydes, and alkylphosphinic acids provides a new, direct, and efficient method for synthesizing Fmoc-protected phosphinic pseudodipeptidic blocks, directly usable for solid-phase peptide synthesis.

Combinatorial chemistry is being increasingly applied for the discovery of novel biologically active compounds. In this context, multicomponent reactions are a powerful tool for the rapid introduction of molecular diversity in terms of lead identification and optimization.[1] The three-component condensation reaction of trivalent phosphorus species, amides, and aldehydes or ketones was first reported in 1974[2] and later extended for the synthesis of α-aminophosphinic and α-aminophosphonic acids.[3] More recently, this type of reaction has been applied in the synthesis of phosphinopeptide analogues of glutathionylspermidine.[4]

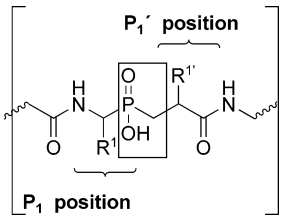


Figure 1. Generic structure of phosphinic peptides.[11a]

Phosphinic peptides (Figure 1) are transition state analogue-type inhibitors of Zn-metalloproteases. Replacement of the scissile peptide bond with the hydrolytically stable P(O)-(OH)CH_2 moiety leads to highly potent and selective inhibitors of various metalloproteases, e.g., angiotensin-converting enzyme (ACE),[5] matrixins (MMPs),[6] aminopeptidase A (APA),[7] leucine aminopeptidase,[8] etc., using either parallel or combinatorial chemistry strategies,[9] both in solution and on solid support.[10]

Resolution of three-dimensional structures of complexes between Zn-metalloproteases and phosphinic peptide inhibi-

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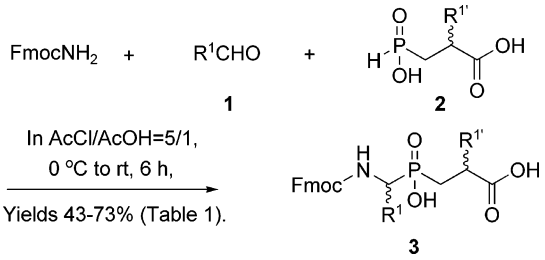
tors has confirmed that these pseudopeptides interact with residues on both sides of the scissile peptide bond.[11b,c] However, such inhibitors should be not only potent but also as selective as possible, to avoid unexpected interactions among multiple zinc metalloproteases, an issue that could be addressed by systematic investigation on the influence of different side chains of the phosphinic inhibitors.

Solid-phase peptide synthesis (SPPS) has been successfully applied in most cases, providing a powerful tool for the development of phosphinic peptide libraries. As far as it concerns the synthesis of the phosphinic pseudodipeptidic block, numerous synthetic strategies have been developed and reviewed,[12] including building block approaches[13] or postmodification of phosphinopeptidic precursors.[14,6a,9a] None of the above-mentioned methods are a panacea for all situations, so new and improved methods are still needed.

In this Letter, we report a new method for the synthesis of phosphinic building blocks, which can be used directly in SPPS. The significant advantages of the present method, as compared to the methods previously described in the literature, are speed and simplicity. Synthesis of the Fmoc-protected phosphinic dipeptidic blocks (Scheme 1) is achieved

Scheme 1. Synthesis of Fmoc-Protected Phosphinic Peptides

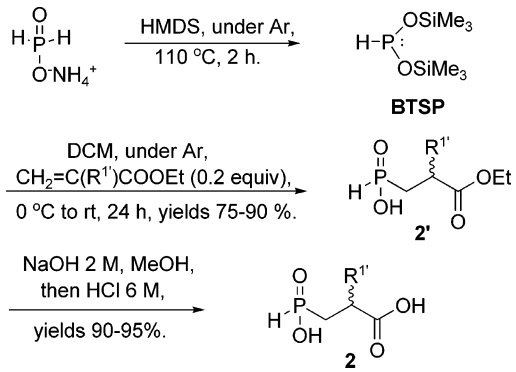


using mostly commercially available reagents, thus avoiding multistep procedures, including laborious protection and deprotection steps.

The basic step for synthesizing phosphinic dipeptidic blocks is the formation of the P−C bond. Generally, this type of reaction requires the activation of the P-moiety to its trivalent form and subsequent attack to an electron-deficient species such as an imine or an acrylic acid ester. In the first case, phosphinic pseudo-amino acids can be formed;[15] phosphinic acids of type **2′** can be formed in the second case.[16] This general idea can find different applications depending on which masking bears the phosphorus moiety.

During the course of this study, we became interested in finding a synthetic pathway in which a masked phosphinic acid of type **2** can attack an imine, thus creating the desired dipeptidic block. The direct use of $FmocNH_2$[17] and phosphinic acids of type **2** is superior to the corresponding method that uses $CbzNH_2$,[18] since the building blocks obtained can be used directly in SPPS. Phosphinic acids of type **2** are synthesized according to reactions depicted in Scheme 2. It

Scheme 2. Synthesis of Compounds Type **2**



is worth mentioning that under the reaction conditions used (5 equiv of bis(trimethylsilyl) phosphonite (BTSP) per 1 equiv of acrylate and high dilution), no disubstituted products were observed. Compounds of type **2** and **2′** are isolated using simple workup procedures, in a pure state, as confirmed by NMR analysis. Acrylic acid esters were either prepared using well-known procedures, e.g., alkylation of malonic acid diethyl ester, selective saponification, and subsequent Knoevenagel condensation (reactions not shown), or were commercially available.

Using this new method, phosphinic peptide building blocks, bearing a variety of R^1 and $R^{1\prime}$ side-chains have been synthesized in yields shown in Table 1. The aldehydes **1** (R^1CHO) bear side-chains (R^1) corresponding to those of some natural amino acids. In this respect, phosphinic peptides with R^1 side-chains of glycine (**3a**), alanine (**3b**), valine (**3c**), leucine (**3d**), isoleucine (**3e**), glutamic acid (**3f**), phenylglycine (**3g**), serine (**3h**), and an analogue of histidine (**3i**) have been synthesized. On the other hand, $R^{1\prime}$ side-chains cor-

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Table 1. Yields and Side-Chains of Compounds **3**

entry	R^1	$R^{1'}$	yields (%)
3a	H	$PhCH_2$	56
3b	CH_3	$PhCH_2$	57
3c	$(CH_3)_2CH$	$PhCH_2$	55
3d	$(CH_3)_2CHCH_2$	$PhCH_2$	62
3e	$CH_3CH_2(CH_3)CH$	$PhCH_2$	61
3f	$CH_3OOCCH_2CH_2$	$PhCH_2$	60
3g	Ph	$PhCH_2$	73
3h	$PhCH_2OCH_2$	$PhCH_2$	67
3i	4-imidazole	$PhCH_2$	69
3j	CH_3	$(CH_3)_2CHCH_2$	58
3k	Ph	CH_3	54
3l	$(CH_3)_2CHCH_2$	H	42

respond to those of phenylalanine (**3a**−**i**), leucine (**3j**), alanine (**3k**), and glycine (**3l**). The yields of pure products are given in Table 1.

Compounds **3b**−**k**, possess two stereogenic centers, namely, four diastereoisomers, two pairs of enantiomers, in approximately the same amount, as indicated by RP-HPLC analyses and ^{31}P NMR. Separation of the four stereoisomers can be achieved by RP-HPLC using a chiral column and the suitable gradient. Alternatively, when blocks **3b**−**k** are incorporated into a peptide sequence, their separation is easily accomplished using a conventional RP column, as shown below in Figure 2 for compound **4**. Though the proposed

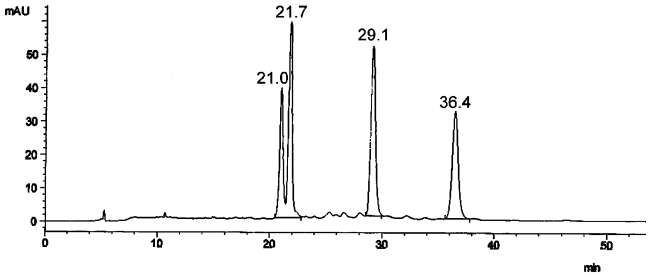
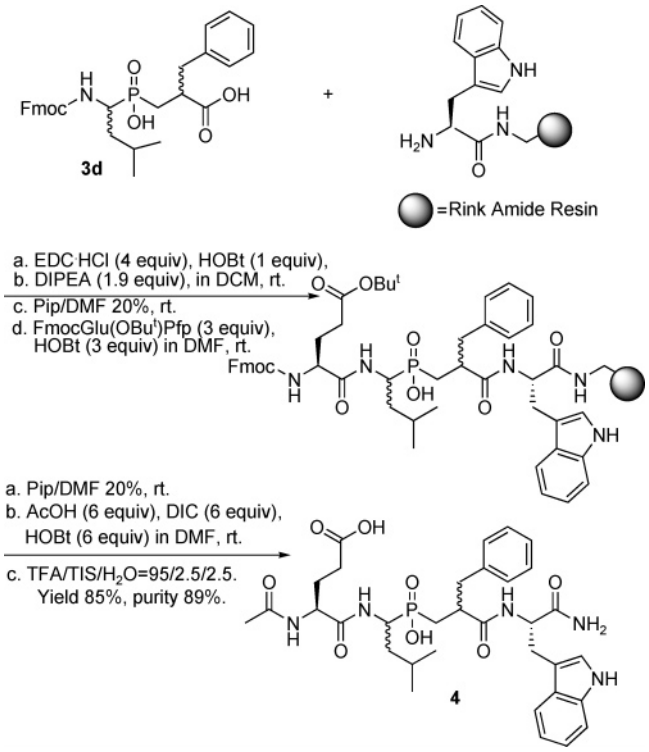


Figure 2. HPLC chromatogram of compound **4**.

synthetic procedure is not stereoselective, it provides the four diastereoisomers of the phosphinic peptide in nearly the same amount, which is convenient, especially when the inhibitory effect of each diasteroisomer is not known, and any of them can be active.

Most of the aldehydes used were commercially available, except those in entries **3f** and **3i**. It is interesting to note that a variety of synthetic methods leading to methyl 4-oxobutanoate (entry **3f**) are reported, including ozonolysis,[19] acetal hydrolysis,[20] oxidation with PCC,[21] reduction with triethylsilane,[22] and modified Rosenmund reduction.[23] None of these

Scheme 3. Solid-Phase Elongation of Phosphinic Peptides



methods gave satisfactory results in our hands with regard to yield and purity of the obtained product, except the original Rosenmund reduction, which provided quantitative and pure product.[24] 4(5)Formylimidazole (entry **3i**) was prepared by oxidation of the corresponding alcohol using MnO_2.[25]

An additional advantage of the present method is the synthesis of phosphinic dipeptides bearing a glutamyl residue in the P_1 position (entry **3f**). As already reported,[14b] Michael-type additions of aspartyl or glutamyl aminophosphinic acids to acrylates fail. Up to now, phosphinic peptides bearing an aspartyl or glutamyl residue in this position were prepared by postmodification using oxidation of a phenyl group to carboxylic acid. Obviously, this method cannot be applied when aryl groups are present in other positions of the phosphinic block. The method proposed here provides a new synthetic route to synthesize such blocks.

In addition, phosphinic peptides containing a glycine residue in the P_1 position (entry **3a**) can be directly synthesized by this method, while the synthesis of the corresponding aminophosphinic acid reported in the literature requires laborious workup and the yield is low.[26]

Finally, a protocol for SPPS elongation of the phosphinic blocks synthesized has been established (Scheme 3). Phosphinic peptides of type **3**, which bear a free carboxylate group, are readily applicable for use in SPPS.

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Although it seems that the use of phosphinic blocks protected at the hydroxyphosphinyl function is a safer choice,[13a,c] utilization of EDC as the coupling reagent ensures short reaction times and lack of byproducts. As shown in Figure 2, all four diasteroisomers of compound **4** can be separated by HPLC.

In conclusion, we have presented a new and very efficient method for the direct synthesis of Fmoc-protected phosphinic building blocks, which can be used directly in SPPS. To our knowledge, it is the first time that $FmocNH_2$ is used in this type of reaction. The reaction is mild and versatile. Most of the reagents used are commercially available. Workup and isolation procedures are simple, and yields are satisfactory. The proposed method can give rise to a large number of combinations between R^1 and $R^{1\prime}$ side chains of phosphinic peptides and allows the synthesis even of capricious sequences.

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Supporting Information Available: Detailed experimental procedures, spectroscopic and analytical data for all compounds, copies of ^1H, ^{13}C, and ^{31}P NMR and MS spectra, and HPLC chromatograms for compounds **3a**−**l**. This material is available free of charge via the Internet at http://pubs.acs.org.

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