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Synthesis of Phosphinopeptides via the Mannich Ligation

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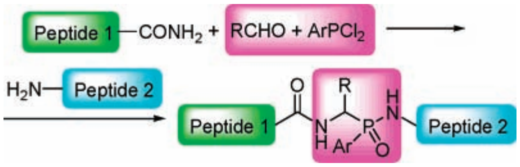
ABSTRACT



Phosphinopeptides are a class of unnatural peptides containing a tetrahedral phosphorus atom and have potential for use as enzyme inhibitors. A series of phosphinopeptides were synthesized via the Mannich-type reaction of aryldichlorophosphines, aldehydes, and N-protected amino amides or peptide amides and subsequent aminolysis with amino esters or peptide esters. The current method named the Mannich ligation is an efficient route to synthesis of phosphinopeptides through convergent condensation.

Phosphonopeptides are a class of unnatural peptides, which contain a tetrahedral phosphorus atom. As the structural analogues of peptides, phosphonopeptides act as antagonists and compete with their carboxylic counterparts for the active sites of enzymes and other cell receptors. They have been widely used as enzyme inhibitors, haptens for production of catalytic antibodies, and antibacterial and herbicidal agents.[1] Phosphonopeptide is a general term describing phosphorus-containing peptides which possess at least one aminoalkylphosphonic acid or aminoalkylphosphinic acid, or even a hydroxyalkylphosphonic acid, in replacement of aminocarboxylic acid(s).[2] Phosphonopeptides generally include phosphonamidopeptides (generally called phosphonopeptides), depsiphosphonopeptides (phosphonatopeptides), and phosphinopeptides (phosphinamidopeptides).[2] Phosphonopeptides are generally prepared via the reaction of aminoalkylphosphonochloridates with amino acid esters or peptide esters and the direct condensation of phosphonate monoesters with amino acid esters or peptide esters.[2] Phosphinopeptides containing a phosphinamide bond have been prepared via the reaction of aminoalkylphosphinic chlorides with amino acid esters or peptide esters,[3] the direct condensation of aminoalkylphosphinic acids and amino esters or peptide esters in the presence of coupling reagents, such as DCC, BOP, and DPPA, with additives HOBt or DMAP in low yields,[4] and the enzyme-catalyzed coupling of ethyl aminoalkylphosphinate and amino esters in satisfactory yields.[5] Herein, we report our approach and results on the synthesis of phosphinopeptides via the Mannich-type condensation and subsequent aminolysis.

Recently, we developed a novel method to synthesize *N*-benzyloxycarbonyl (*Z*)-protected phosphonopeptides and depsiphosphonopeptides via the Mannich-type condensation

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[1] (a) For a review, see: Kafarski, P.; Lejczak, B. In *Aminophosphonic and Aminophosphinic Acids: Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; John Wiley: Chichester, 2000; Chapter 12, pp 404−442. (b) For a recent example, see: Ravaschino, E. L.; Docampo, R.; Rodriguez, J. B. *J. Med. Chem.* **2006**, *49*, 426−435.

[2] For a review, see: Kafarski, P.; Lejczak, B. In *Aminophosphonic and Aminophosphinic Acids: Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; John Wiley: Chichester, 2000; Chapter 6, pp 173−204.

[3] (a) Moree, W. J.; van der Marel, G. A.; van Boom, J. H.; Liskamp, R. M. J. *Tetrahedron* **1993**, *49*, 11055−11064. (b) Mucha, A.; Kafarski, P.; Plenat, F.; Cristau, H.-J. *Tetrahedron* **1994**, *50*, 12743−12754.

[4] Elhaddadi, M.; Jacquier, R.; Petrus, C.; Petrus, F. *Phosphorus Sulfur Silicon* **1991**, *63*, 255−259.

[5] Natchev, I. A. *Tetrahedron* **1991**, *47*, 1239−1248.

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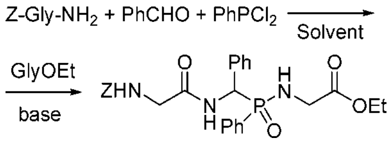
of benzyl carbamate, aldehydes, and dichlorophosphites and subsequent aminolysis with amino acid esters or alcoholysis with hydroxy acid esters, respectively;[6] depsiphosphonopeptides were synthesized via the Mannich-type condensation of benzyl carbamate, aldehydes, and mono/dichlorophosphites, followed by subsequent hydrolysis.[7] However, the method can only be used to synthesize (depsi)phosphonopeptides with N-terminal aminoalkylphosphonic acids because the Mannich-type reaction does not work when amino amides are used instead of benzyl carbamate possibly due to the weak nucleophilicity of amides and the weak dehydrating ability of chlorophosphites. The Mannich-type reaction of benzyl carbamate, aldehydes, and phenyldichlorophosphine in acetic acid or in acetyl chloride and subsequent hydrolysis were used previously to prepare *N*-*Z*-aminoalkylphosphinic acids.[8] The Mannich reaction in acetyl chloride and subsequent alcoholysis and aminolysis were mentioned as being used to prepare alkyl *N*-*Z*-aminoalkylphosphinates and *N*-*Z*-aminoalkylphosphinamides, and even *N*-*Z*-protected phosphinodipeptides, via aminolysis with ethyl glycine.[9] However, no characterization data of these products were reported and no experimental procedure was provided for the preparation of phosphinodipeptides.[9] Even if this reaction works, it could only be used to synthesize phosphinopeptides with *N*-*Z*-aminoarylmethylphosphinic acids at the N-terminus, not in the middle position of the phosphinopeptides. The same group also reported that the Mannich-type reaction of *p*-toluenesulfonamide, benzaldehyde, and phenyldichlorophosphine in acetyl chloride and subsequent aminolysis with glycine ester were used to prepare a phosphinodipeptide with *N*-tosylaminophenylmethylphosphonic acid at the N-terminus.[10] The solvent acetyl chloride was used as an efficient dehydrating agent.

We hoped to develop a general and practical method to synthesize phosphinopeptides, in which the aminoalkylphosphinic acid is located in the middle position of the peptides. Thus, we need to use an amino amide or peptide amide instead of the sulfonamide or carbamates. Amino amides are poorer nucleophiles than benzyl carbamate. We assumed that phenyldichlorophosphine would be a stronger dehydrating agent than chlorophosphites and would be well suited to drive the Mannich-type reaction involving amides and thus could be used to prepare phosphinopeptides. First, we selected the reaction of *N*-benzyloxycarbonyl (*Z*)-glycinamide, benzaldehyde, and phenyldichlorophosphine, followed by subsequent aminolysis with ethyl glycine as a model reaction for method optimization. The results are summarized in Table 1.

Table 1. Optimizing Reaction Conditions for Synthesis of Phosphinopeptide from *N*-*Z*-Glycinamide, Benzaldehyde, Phenyldichlorophosphine, and Ethyl Glycine[a]



entry	solvent	base/amount (equiv)	PhPCl$_2$ (equiv)	temp (°C)	yield (%)
1	toluene	TEA (5)	2	rt	–
2	$CHCl_3$	TEA (5)	2	rt	–
3	DMF	TEA (5)	2	rt	–
4	DMSO	TEA (5)	2	rt	–
5	CH_2Cl_2	TEA (5)	2	rt	17
6	MeCN	TEA (5)	2	rt	36
7	MeCN	TEA (6)	3	rt	35
8	MeCN	TEA (5)	2	50	30
9	MeCN	TEA (5) DMAP (0.2)	2	rt	32
10	MeCN	TEA (5) 4 Å MS (0.2)	2	rt	trace
11	MeCN	TEA (5)	2	rt	27[b]
12	MeCN	DIEA (5) DMAP (0.2)	2	rt	47
13	MeCN	DIEA (5)	2	rt	65
14	MeCN	DIEA (4)	1.17	rt	67
15	MeCN	DIEA (5)	1.17	rt	47[c]

[a] Reaction was carried out in a 3 mmol scale of *N*-*Z*-glycinamide under a nitrogen atmosphere with 6 mmol of ethyl glycine in aminolysis. [b] Reaction was conducted without nitrogen protection. [c] Aminolysis with ethyl glycine hydrochloride.

We looked to replace acetyl chloride with an inert solvent to enable a one-pot methodology. Initially, we tried the reaction in toluene, but no reaction occurred because of the poor solubility of *N*-*Z*-glycinamide (Table 1, entry 1). To increase the solubility, we tried an array of solvents including chloroform, dichloromethane, acetonitrile, DMF, and DMSO. No desired product was obtained in chloroform, DMF, and DMSO, most likely due to the reaction between phenyldichlorophosphine and the solvents (Table 1, entries 2−4). The desired product was obtained in low yields in dichloromethane and acetonitrile (Table 1, entries 5 and 6). With an increasing amount of triethylamine (TEA) (Table 1, entry 7), increasing reaction temperature (Table 1, entry 8), and addition of additive 4-dimethylaminopyridine (DMAP), a widely used catalyst in acylation (Table 1, entry 9), no further improvement in the yield was achieved. Even in the presence of 4 Å molecular sieves, only trace amounts of product were obtained (Table 1, entry 10). It was reported that TEA could react with phosphinic chloride to yield a phosphinyl triethylammonium ion,[3b] which may affect the yield of the desired product. To inhibit this side reaction, we used more bulky diisopropylethylamine (DIEA) instead of TEA as the base. By decreasing the ratios of DIEA and phenyldichlorophosphine, 4 equiv and 1.17 equiv, respectively, to glycinamide (Table 1, entry 14), the yield improved from 36% to 67%. Ethyl glycine hydrochloride also showed lower reactivity than free ethyl glycine (Table 1, entries 14 and 15). As usual workup in the peptide synthesis, phosphinopeptides were purified by sequential washing with saturated sodium bicarbanate and 0.02 mol/L of citric acid aqueous solution to

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(6) Fu, N. Y.; Zhang, Q. H.; Duan, L. F.; Xu, J. X. *J. Pept. Sci*. **2006**, *12*, 303−309.

(7) (a) Xu, J. X.; Gao, Y. H. *Synthesis* **2005**, 783−788. (b) Liu, H.; Cai, S. Z.; Xu, J. X. *J. Pept. Sci*. **2006**, *12*, 337−340.

(8) (a) Oleksyszyn, J. *Synthesis* **1980**, 722−724. (b) Dai, Q.; Chen, R. Y. *Synthesis* **1997**, 415−416.

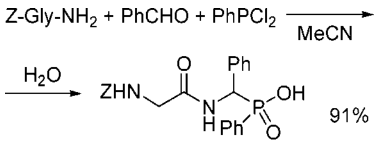
(9) Dai, Q.; Chen, R. Y. *Synth. Commun* **1997**, *27*, 17−22.

(10) Dai, Q.; Chen, R. Y. *Chin. J. Chem*. **1997**, *15*, 283−285.

remove non-aminolytic phosphinic chloride and excess amino ester, respectively. Our experimental results indicate that phosphinopeptides are stable in 0.02 mol/L of citric acid solution and even in 0.1 mol/L for some phosphinopeptides. For the optimized procedure, phenyldichlorophosphine was added to a solution of *N*-*Z*-glycinamide and benzaldehyde in dry acetonitrile under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 12 h and further stirred for 24 h after addition of DIEA and ethyl glycine. The current route is an efficient method for the synthesis of phosphinopeptides in satisfactory yields when compared with previous methods.[3−5]

To identify which step controls the total yield, we studied the reaction of *N*-*Z*-glycinamide, benzaldehyde, and phenyldichlorophosphine, followed by subsequent hydrolysis (Scheme 1). The dipeptide was obtained in a total yield of 91%. This indicates that it is the aminolysis step that limits the total yield in the phosphinopeptide synthesis. The current method is an efficient route to prepare phosphinopeptides with C-terminal aminoalkylphosphinic acids via the convergent synthesis.

Scheme 1. Synthesis of Phosphinopeptide with C-Terminal Aminoalkylphosphinic Acid

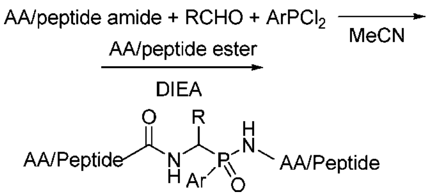


After successful synthesis of phosphinopeptide derived from *N*-*Z*-glycinamide, benzaldehyde, phenyldichlorophosphine, and ethyl glycine, we extended the method to prepare a series of phosphinopeptides, including two phosphinotetrapeptides. It should be noted that both aromatic and aliphatic aldehydes work efficiently in the current method. Phenyldichlorophosphine could be replaced with other aryldichlorophosphines (Table 2, entries 2 and 3). In addition, *N*-*Z*-glycinamide could be replaced with other *N*-*Z*-protected amino amides, even the *N*-*Z*-peptide amide (Table 2, entry 15). Ethyl glycine was extended to other amino esters, even the peptide ester (Table 2, entry 13). Methyl L-phenylalanine hydrochloride and ethyl glycinylglycine hydrochloride also show lower reactivity than the corresponding free amino or peptide esters (Table 2, entries 11 and 14). Phosphinotetrapeptides were obtained in relatively low yields because of poor solubility of glycinylglycine amide and ethyl glycinylglycine.

For aminolysis with peptide esters, ethyl glycinylglycine was first dissolved in acetonitrile in the presence of DIEA and then added into the reaction mixture, thus compensating for the poor solubility of ethyl glycinylglycine.

The results indicate that the current method could be used to synthesize oligomeric phosphinopeptides via a pseudo-four-component condensation, that is, Mannich-type three-component condensation of an amide, aldehyde, and aryldichlorophosphine and subsequent aminolysis with an amino ester/peptide ester. Because the current method can link two peptide segments in mild conditions via the Mannich-type reaction as the key step, we named it the Mannich ligation. Ligation has been widely used to prepare peptides and proteins during the last two decades.[11]

Table 2. Synthesis of Phosphinopeptides via the Mannich Ligation[a]



entry	amide	aldehyde	Ar	ester	yield (%)
1	*Z*-Gly-NH$_2$	PhCHO	Ph	GlyOEt	67
2	*Z*-Gly-NH$_2$	PhCHO	*p*-MePh	GlyOEt	68
3	*Z*-Gly-NH$_2$	PhCHO	*p*-ClPh	GlyOEt	71
4	*Z*-Gly-NH$_2$	*p*-MePhCHO	Ph	GlyOEt	70
5	*Z*-Gly-NH$_2$	*p*-ClPhCHO	Ph	GlyOEt	65
6	*Z*-Gly-NH$_2$	^{i}PrCHO	Ph	GlyOEt	69
7	*Z*-Gly-NH$_2$	^{n}PrCHO	Ph	GlyOEt	69
8	*Z*−D/L-Ala-NH$_2$	PhCHO	Ph	GlyOEt	64[c]
9	*Z*-β-Ala-NH$_2$	PhCHO	Ph	GlyOEt	60
10	*Z*-Gly-NH$_2$	PhCHO	Ph	L-PheOMe	68[d]
11	*Z*-Gly-NH$_2$	PhCHO	Ph	L-PheOMe HCl	trace
12	*Z*-Gly-NH$_2$	PhCHO	Ph	β-Ala OEt	69
13	*Z*-Gly-NH$_2$	PhCHO	Ph	GlyGlyOEt	51
14	*Z*-Gly-NH$_2$	PhCHO	Ph	GlyGlyOEt HCl	28
15[b]	*Z*-GlyGly-NH$_2$	PhCHO	Ph	GlyOEt	54

[a] Reaction was conducted in a 3 mmol scale of *N*-*Z*-amino amide under a nitrogen atmosphere with a ratio of amide/aldehyde/PhPCl$_2$ of 3:3.5:3.5 at rt for 12 h first and then stirred for 24 h after addition of DIEA (12 mmol) and the ester (6 mmol). [b] Reaction was conducted at 50 °C for 6 days first and then stirred for 24 h after addition of DIEA and the ester. [c] No diastereoselectivity was observed on the basis of ^{31}P NMR (only one peak in ^{31}P NMR) possibly because the chiral carbon atom is far away from the reacting center. [d] Ratio of (*S*,*S*,*S*)-diastereomer/(*R*,*R*,*S*)-diastereomer is 1.07:1.00 on the basis of the integral of peaks at 30.2 and 29.7 ppm in the ^{31}P NMR spectrum.

As for the reaction mechanism, it was assumed that an amide first attacks an aldehyde to form an *N*-acylaminohydrin adduct, which further reacts with an aryldichlorophosphine to produce an arylchlorophosphite. The phosphite undergoes an elimination to give rise to an imine and an arylchlorophosphonous acid. The arylchlorophosphonous acid then attacks the imine, and subsequent proton transfer

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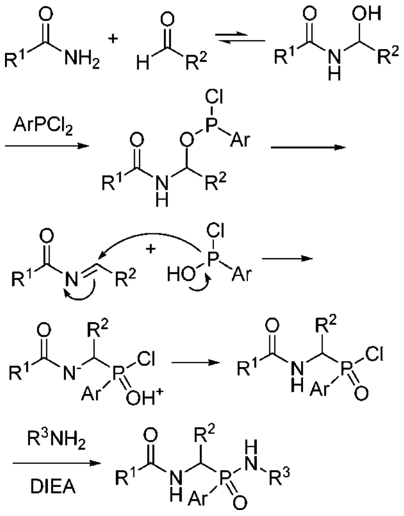
(11) For recent reviews, see: (a) Tam, J. P.; Xu, J. X.; Eom, K. D. *Biopolymers (Pept. Sci.)* **2001**, *60*, 194−205. (b) Ma, Y.; Zhao, Y. F. *Chin. Sci. Bull.* **2003**, *48*, 1−4. (c) Ma, Y.; Zhao, Y. F. *Chem. Prog.* **2003**, *15*, 393−400. (d) Bode, J. W. *Curr. Opin. Drug Discovery Dev.* **2006**, *9*, 765−775. (e) Brik, A.; Ficht, S.; Wong, C. H. *Curr. Opin. Chem. Biol.* **2006**, *10*, 638−644. For recent examples, see: (f) Pentelute, B. L.; Kent, S. B. H. *Org. Lett.* **2007**, *9*, 687−690. (g) Soellner, M. B.; Tam, A.; Raines, R. T. *J. Org. Chem.* **2006**, *71*, 9824−9830. (h) Macmillan, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 7668−7672. (i) Dirksen, A.; Meijer, E. W.; Adriaens, W.; Hackeng, T. M. *Chem. Commun.* **2006**, 1667−1669. (j) Lu, Y. A.; Tam, J. P. *Org. Lett.* **2005**, *7*, 5003−5006.

Scheme 2. Proposed Mechanism for the Mannich Ligation



affords the key intermediate as the aminoalkylphosphinic chloride, which undergoes an aminolysis with an amino ester or peptide ester to generate the final product phosphinopeptide (Scheme 2).

In summary, a new method named the Mannich ligation was developed for synthesis of phosphinopeptides via the Mannich-type reaction of aryldichlorophosphines, aldehydes, and *N*-benzyloxycarbonyl-protected amino amides or peptide amides and subsequent aminolysis with amino esters or peptide esters. A series of phosphinopeptides were synthesized in satisfactory to good yields in a one-pot reaction. The method can also be used to prepare phosphinopeptides with C-terminal aminoalkylphosphinic acids in good yields. The current method is an efficient and convenient route to synthesis of phosphinopeptides.

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Supporting Information Available: Experimental procedure, the analytical data, and copies of ^{1}H NMR and ^{13}C NMR spectra of phosphinopeptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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