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An expedient synthesis of peptidyl *N*-alkylamides by ''HOPE'' strategy

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Abstract

We have developed an expedient approach, ''HOPE'' (hybrid orthogonal protocol with ease) strategy for the synthesis of peptidyl *N*-alkylamides. This new strategy was characterized by following points: incorporating Boc and Fmoc protocols together on Merrifield resin, removal of SPG (side-chain protecting groups) without the damage of linker structure on the resin, and the ammonolysis of linker as the last step could achieve the introducing *N*-alkylamide structure into *C*-terminal and releasing product from resin-support simultaneously. In present work, eight peptidylamides with different alkylsubstitution at *C*-terminal were conveniently synthesized by HOPE strategy.

Keywords: ''HOPE'' strategy; Solid-phase peptide synthesis; Peptidyl *N*-alkylamide

Many C-terminal modified peptides are used as active pharmaceutical ingredients (APIs), such as leuprolide [1], DAGO [2], octreotide [3], and some of them have been applied in clinic for years. Also some *C*-terminally mutant peptides are found in nature and have potential interest as therapeutic agents [4]. Usually, it is difficult to prepare *C*-terminal modified peptide by conventional solid-phase peptide synthesis (SPPS), because the final products released from resin always have a COOH or $CONH_2$ structure at *C*-terminal. To our knowledge, some labs have been engaged in the synthesis of peptide *N*-alkyl amides, and the general ideas they focused were as follows: (1) total liquid-phase synthesis [5]; (2) Boc protocol on Merrifield resin followed by ammonolysis and HF deprotection [6]. (3) Fmoc protocol on aryl hydrazide resin followed by ammonolysis and TFA deprotection [7]; (4) Fmoc protocol on 2-Cl-Trt resin followed by liquid-phase coupling of *C*-terminal carboxylic acid (liberated from Trt resin by 5% TFA-DCM) with *N*-alkyl amine [8,13]; (5) Fmoc protocol on *N*-alkyl linker resin followed by photolysis or TFA cleavage [9–11].

It is obvious that some protocols related in references are probably not suitable for large scale production by following points: (A) SPG (side-chain protecting group) deprotection by HF treatment in Boc protocol; (B) additional liquid-phase coupling of protected peptide *C*-terminal carboxylic acid with *N*-alkyl amines; or (C) special linker resins with limited *N*-alkyl substitution were used instead of case B. Hence, there is a need to develop a more practicable approach to the on-resin synthesis of *C*-terminal modified peptides. We envisaged that the key points in this new

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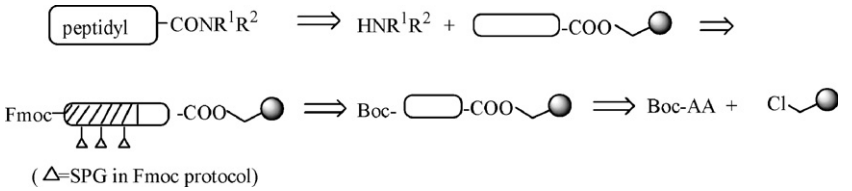
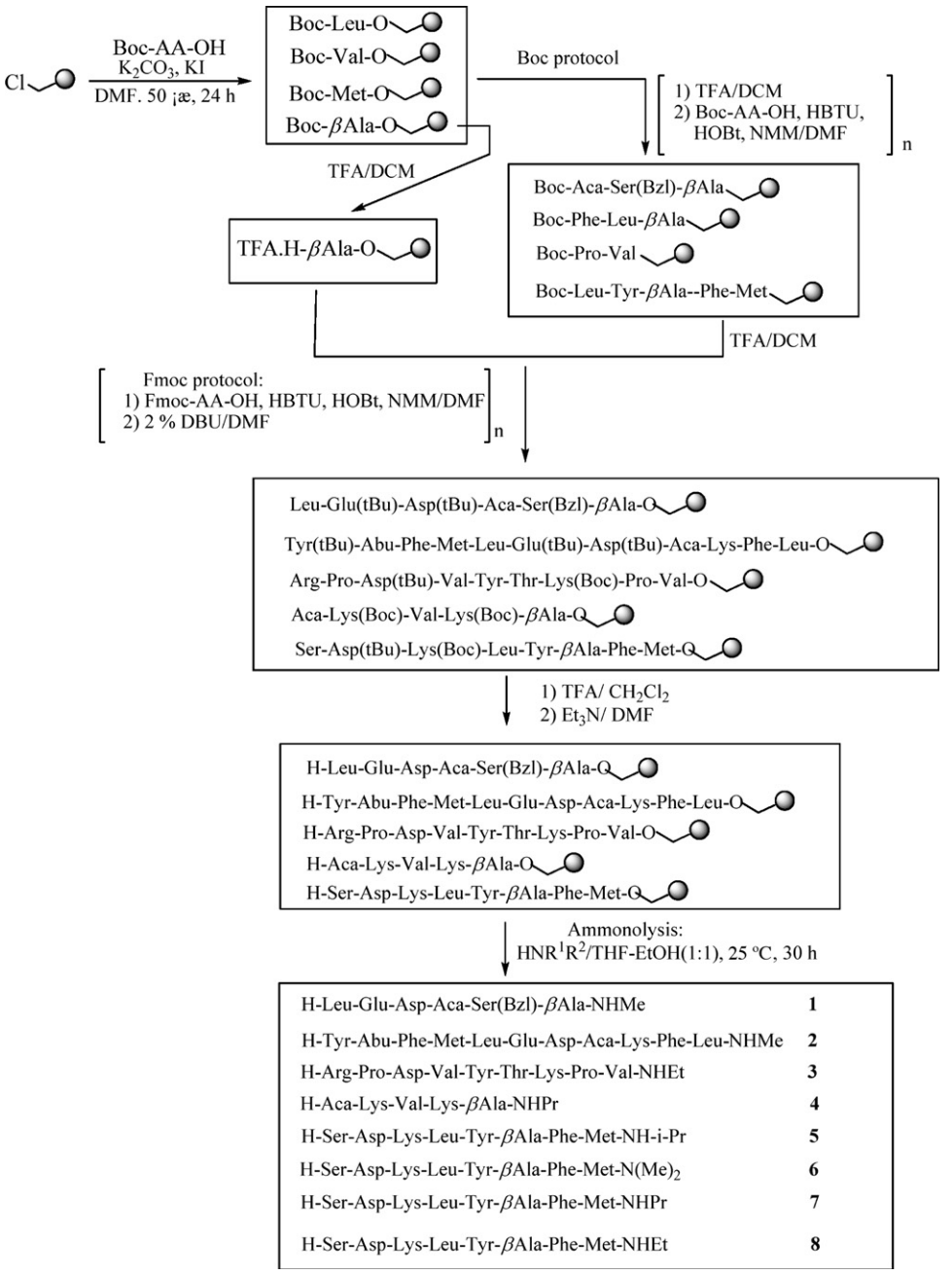


Scheme 1. Retrosynthetic route of peptidyl *N*-alkylamide.



Scheme 2. Synthesis of peptidyl *N*-alkylamide.

approach should be: (1) the incorporation of Fmoc and Boc protocol on Merrifield resin together; (2) keeping the benzyl ester linker integrity during SPG deprotection; (3) Fmoc protocol must be applied for the sake of all SPGs should be removed without the damage to resin linker; (4) ammonolysis as the multi-functional step for introducing *N*-alkyl amide structure and liberating product from resin simultaneously. We would like to represent this new approach in brief terms of HOPE strategy based on the feature: hybrid orthogonal protocol with ease.

In present work, the synthesis of eight peptidyl amides with different *N*-alkyl substitution was disposed based on the following retrosynthetic analysis (Scheme 1).

For HOPE strategy, Merrifield resin was our prior choice of solid support, because of the benzylester linkage was stable to TFA in the course of eliminating α-*N*-Boc group and SPGs related in Fmoc protocol, and easy to be ammonolized by amine to release the targeted product: peptidyl *N*-alkylamide.

A crucial point in HOPE strategy is all SPGs must be removed by TFA condition without the damage to benzylester linker on the resin. Therefore, Fmoc protocol would be qualified and be disposed only after Boc protocol. In other words, some residues with chemically inert side-chain, such as Ala, Gly, Ile, Leu, Phe, Pro and so on, could be assembled before the performance of Fmoc Protocol by Boc protocol if they are located in the region of C-terminal (Scheme 2).

In present synthesis, some non-proteinogenic residues, such as Aca (6-aminocaproic acid) and β-Ala were introduced for products **1**, **2** and **4**–**8**, acting as a form of structure modification. Different amines were used in ammonolysis aimed at enhancing *C*-terminal structural diversity. In order to understand the different yields of ammonolysis with different blocking amines, compounds **5**–**8** were designed, they had the same sequence but different alkyl substitutions of the *C*-terminal. The overall yield of products was calculated by integrating the yield of sequential assembly on solid-support based on the resin-weight increment and the yield of ammonolysis (Table 1).

From Table 1, it is evident that the yields of crude products are dependent mainly on the yields of ammonolysis. The yields of product **1**–**4**, **7** and **8** are quite reasonable. Moderate yields and low purity of peptides **5** and **6** were obtained and indicated the effect of stereohindrance from isopropyl and dimethyl groups on N atom was conspicuous.

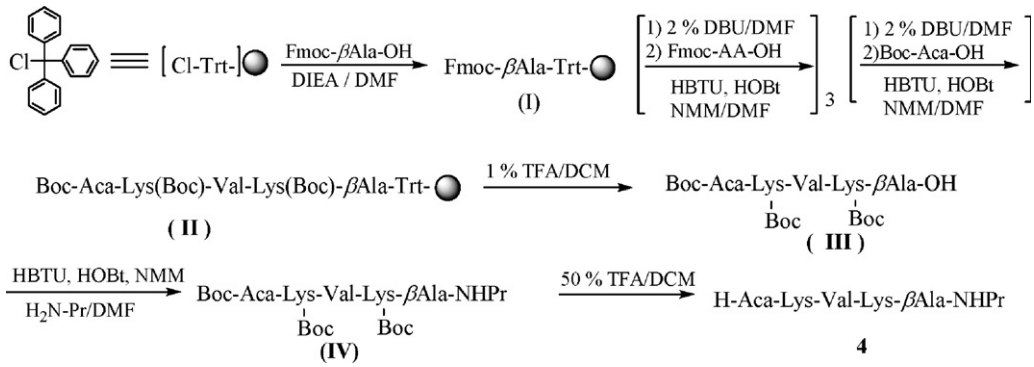
For comparing HOPE strategy with conventional strategy, peptide **2** and **4** were also prepared by standard Fmoc protocol [12], the synthetic route of compound **4** was presented (Scheme 3).

Table 1
The yields, purity and ESI-MS analysis of compound **1**–**8**.

Compound	*C*-terminal structure	Yields of sequential assembly	Yields of ammonolysis	Overall yields of crude products	Purity before HPLC separation	Purity after HPLC separation	Overall yields of final products	Calculated molecular weight	ESI *m/z* obsd.
1	CONHMe	87.9%	95.1%	83.6%	85.0%	97.65%	62.9%	877.49	878.4981 MH^+
2	CONHMe	95.5%	96.2%	91.9%	94.2%	98.72%	74.5%	1415.75	1416.7555 MH^+
3	CONHEt	79.2%	96.0%	76.0%	78.5%	96.64%	51.7%	1100.63	1101.6226 MH^+
4	CONHPr	91.1%	93.6%	85.5%	70.2%	95.46%	47.8%	598.45	599.4687 MH^+
5	CONHi-Pr	80.5%	50.6%	40.7%	65.7%	94.73%	21.3%	1014.52	1015.5132 MH^+
6	$CONHMe_2$	80.5%	71.5%	57.5%	55.7%	93.25%	27.8%	1000.51	1001.4449 MH^+
7	CONHPr	95.7%	93.5%	89.5%	76.7%	94.61%	57.3%	1014.52	1015.5157 MH^+
8	CONHEt	97.0%	95.8%	93.0%	81.8%	95.87%	64.6%	1000.51	1001.5009 MH^+

Table 2
Benefit comparison between HOPE and Fmoc protocol in the synthesis of products **2** and **4**.

Protocol Resin		Fmoc protocol Cl-Trt-	HOPE protocol Merrifield
Liquid-phase reaction		2	0
Coupling steps	**2**	11	10
	4	9	8
Yields of amine introduced to *C*-terminal	**2**	86.3%	96.2%
	4	85.1%	93.6%
Total yields of crude product	**2**	81.9%	91.9%
	4	71.6%	85.5%



Scheme 3. Synthesis of peptidyl *N*-alkylamide compound **4** by Fmoc strategy.

It was obvious that two extra-steps, coupling with propylamine and deprotection with TFA in solution phase, were indispensable in conventional Fmoc strategy. Compared with HOPE strategy, standard Fmoc protocol was more tedious and lower yield (Table 2).

In summary, we have developed HOPE strategy for solid-phase synthesis of peptidyl *N*-alkylamide. This new strategy was mainly characterized by the incorporation of Fmoc/Boc chemistry on Merrifield resin, removal of SPGs without the damage of linkage and the ammonolysis of linker at the last step to achieve both the assembling of *C*-terminal *N*-alkylamide and releasing final product from resin support simultaneously. In view of being more feasible and more economical, HOPE strategy would be a reasonable way to synthesize *C*-terminal modified peptides, especially peptidyl *N*-alkylamides.

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