Chinese Chemical Letters Vol. 16, No. 1, pp 31-34, 2005 31
http://www.imm.ac.cn/journal/ccl.html

Short Synthesis of C-terminal Modified Peptides by a Series-connection Procedure

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Abstract: Three peptide alcohols and four peptidyl N-akyl-amides were prepared by a series-connection procedure consisting of n-1 sequencial assembly on solid support followed by ammonolysis with glycinol, benzylamine or *n*-butylamine, and successive extractionelution through C-18 layer. All products were obtained from this procedure without further purification, in an overall yield of 75-86%.

Keywords: Ammonolysis, C-18 filtration, solid-phase synthesis.

Most bioactive peptides with poor bioavailability, short duration of action and lack of oral activity always limit their use in clinic. Therefore, structure modification of peptides would be very important for developing valuable leading compounds. Much efforts have been towards the preparation of locally or globally modified peptide analogues. Octreotide[1] and DAGO[2] both with hydroxy group at C-terminal were the example of introducing non-proteinogenic structural unit to peptide molecules.

To our knowledge, there were three different methods for peptidyl alcohol preparation by solid-phase method in related papers: (I) reductive cleavage of peptidyl ester linker[3], (II) standard cleavage of peptidol-ester linker[4-6], and (III) ammonolytic cleavage of peptidyl ester linker[7]. Recently, we have been focusing our attention to the synthesis of locally modified osteogenic growth peptide fragments[8] using amino ethanol (glycinol, Gol), $BuNH_2$ and $BnNH_2$ as the ammonolytic reagents to peptidyl ester-bonded resin.

All target compounds were pentapeptides including a modified C-terminal residue, which was incorporated at the ammonolytic cleavage stage, so that only four residue sequence of pentapeptide should be assembled on the resin. That was so called n-1 sequential assembly. According to this strategy four tetrapeptidyl resins (**1-4**) were assembled as the intermediates for sevenfinal compounds (**5-11**) **(Scheme 1)**.

In general, there is not any difficult to isolate the final product after the cleavage of peptidyl ester linked resin with NH_3, $MeNH_2$ or $EtNH_2$. In the case of using amino alcohol or some amines, which possess higher boiling point and the solubility similar to

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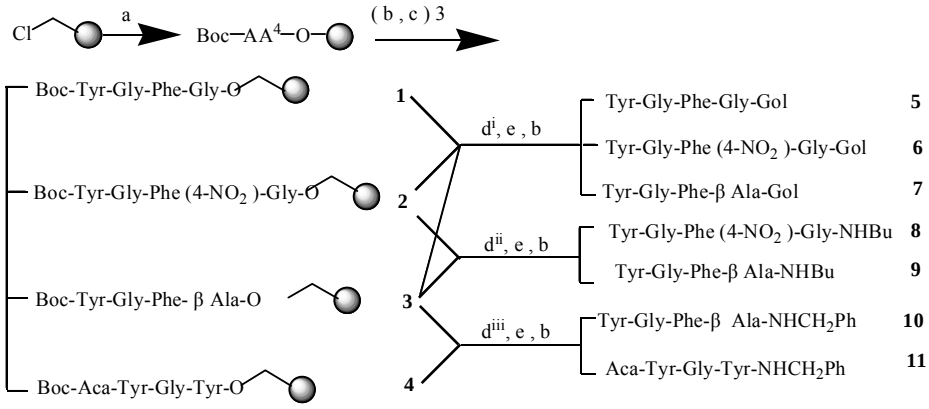
the product, as the amino component in ammonolytic cleavage, the elimination of excess amino component from final product is always troublesome. In view of this situation, a solid phase extraction-elution treatment on C-18 layer was disposed in our lab (**Figure 1**). After C-18 filtration, Boc protected product was treated with HCl/HOAc providing final product.

Based on this procedure, three peptide alcohols (**5, 6, 7**), two peptidyl N-butyl-amides (**8, 9**) and two peptidyl N-benzylamides (**10, 11**) were obtained conveniently in pretty good overall yields, and all products were confirmed by amino-acid analysis and FAB-MS **(Table 1).**

Scheme 1



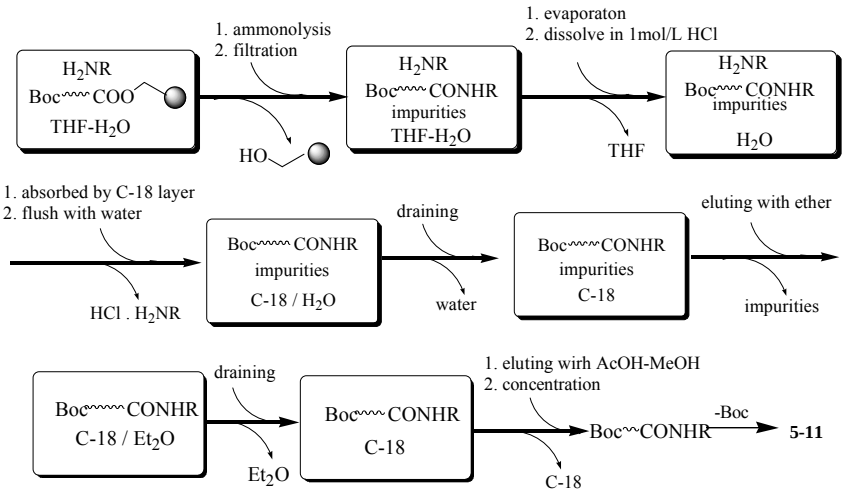
Boc-AA^4-OH, Cs_2CO_3, NaI (cat.)/ DMF, 60℃; (b) 3.5 mol/L HCl/HOAc; (c) Boc-AA^3-OH (Boc-AA^2-OH, Boc-AA^1-OH), DCC, HOBt, DIEA/ DMF; (d) H_2NR (R^i = C_2H_4OH, R^{ii} = *n*-Bu, R^{iii} = Bn)/ THF-H_2O (9:1), 24 h; (e) C-18 filtration

Table 1 Yields and structure confirmation data for **5-11**

Compound	Structure	Yield (%)	AAA[a]	FAB-MS (M+1)
5	Tyr-Gly-Phe-Gly-Gol	81	Tyr 0.98 (1), Gly 2.03 (2), Phe 1.00 (1)	486.2
6	Tyr-Gly-Phe (4-NO_2)-Gly-Gol	78	Tyr 0.99 (1), Gly 2.03 (2)	531.4
7	Tyr-Gly-Phe- β Ala-Gol	86	Tyr 0.99 (1), Gly 1.01 (1), Phe 1.01(1), β Ala1.02 (1)	500.3
8	Tyr-Gly-Phe (4-NO_2)-Gly-NHBu	75	Tyr 0.98 (1), Gly 2.03 (2)	577.3
9	Tyr-Gly-Phe- β Ala-NHBu	83	Tyr 0.98 (1), Gly 1.01 (1), Phe 1.00 (1), β Ala1.03 (1)	512.2
10	Tyr-Gly-Phe- β Ala-$NHCH_2Ph$	80	Tyr 0.98 (1), Gly 1.01 (1), Phe 1.01 (1), β Ala 1.02 (1)	546.4
11	Aca-Tyr-Gly-Tyr-$NHCH_2Ph$	76	Aca 0.98 (1), Tyr 1.97 (2), Gly1.02 (1)	604.3

a Automated amino acid analysis

Figure 1 Solid phase extraction-elution procedure



In conclusion, we have demonstrated a short synthesis of C-terminal modified peptides, involving a n-1 sequential assembly on solid support followed by ammonolysis to incorporate a modified C-terminal residue and releasing the product from resin simultaneously. Compared to sephadex filtration, C-18 layer could be flushed with large amount of water to exhaustively eliminate HCl.H_2NR and other water-soluble small molecules. Compared to HPLC purification, C-18 layer filtration related in this paper was more concise, economical and feasible for large scale purification. On the other hand, we should noticed that if the purity of the crude product was very poor (including many deletion peptides), and some products with a MW below 500, C-18 layer filtration would be failed to purify these products, due to its poor affinity to C-18 particles.

General Procedure

*The synthesis of intermediates **1-4***

For each synthesis, the chloromethyl resin (1.3 g, 1 mmol) was mixed with Boc-AA^4-OH (3 mmol) , Cs_2CO_3 (1.5 mmol), NaI (0.1 mmol) and DMF (10 mL). The mixture was stirred at 60℃ for 24 h. After draining and washing successively with DMF (×3), MeOH (×2), DMF (×2), EtOH (×3), and DCM (×2), a standard solid-phase peptide coupling-cycles were performed on the Boc-AA^4-O-resin.

Releasing crude products by ammonolysis.

The tetrapeptidyl resin (1 mmol) and amino component (10 mmol)(aminoethanol for **5, 6, 7**; *n*-butylamine for **8, 9** and benzylamine for **10, 11**) were mixed with 15 mL of 90%THF/H_2O in a sealed tube. The mixture was shaken at r.t. for 24 h. The supernatant was collected and concentrated *in vacuo* to dryness.

C-18 layer filtration

The syrupy crude product (~1 mmol) was dissolved in 100 mL of 1 mol/L HCl / H_2O and filtered through C-18 layer [30 (h) × 40 (φ) mm] in a sintered glass funnel. The C-18 layer was then flushed with plenty of water until the result of ninhydrin test with drops of filtrate was negative. After draining off water, the dried C-18 layer was washed with ether (~100 mL). The product adsorbed in C-18 layer was eluted with AcOH-MeOH (1: 2), monitored by ninhydein test on drops of eluate. The eluate was concentrated in vacuum at 50℃ to dryness.

*Deprotecting-Boc treatment providing products **5-10***

Dried residue of Boc-peptidyl amide (~1mmol) was mixed with 10 mL of 3.5 mol/L HCl / HOAc at 0℃, stiring for 1 h and then at r.t. for 6 h. The mixture was concentrated in vacuum at 45℃ to dryness. The process of adding toluene (50 mL) and concentrating to dryness was repeated three times. To the dried residue anhydrous ether (30 mL) was added. After triturating and filtrating, the powdered product was obtained (yield 75~86%).

Acknowledgments

This work was supported by the National Natural Science Foundation of China, grants No. 30271530.

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Received 8 December, 2003