Chinese Chemical Letters Vol. 13, No. 12, pp 1154 – 1157, 2002
http://www.imm.ac.cn/journal/ccl.html

An Improved Synthesis of Laminin Fragment CR9

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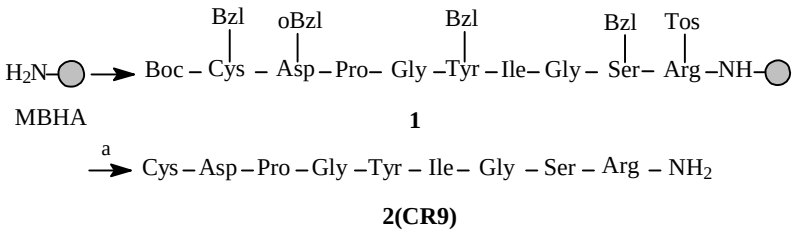
Abstract: Laminin nonapeptide CR9 was synthesized *via* two different methods. A notably enhanced yield (46.8%) was obtained from method B compared to that (12.4%) from standard protocol (method A).

Keyword: Solid-phase peptide synthesis, MBHA resin, Pac resin.

In solid-phase synthesis, the protection-cleavage strategy is strictly determined by the linker structure which has been anchored on the resin support before the assembly of targeted compound. In order to develop a more feasible and economical process of synthesizing nonapeptide CR9, H-Cys-Asp-Pro-Gly-Tyr-Ile-Gly-Ser-Arg-NH_2, which is known having very high affinity to breast tumor[1] and can be radiolabeled as potential diagnostic agent for metastatic cancers[2], two different synthetic methods were tested in present study.

A commercially available solid support MBHA (4-methylbenzhydrylamine) resin[3] was used in method A. The Boc/Bzl protection and HF cleavage were used to match MBHA linker. In method B, the bromoacetyl polystyrene resin[4], which has a phenacyl (Pac) linker, was the solid support. Considering Pac-linker is generally cleaved by HF or Hi-TFMSA procedure[5], and is quite stable to Lo-TFMSA condition, a new protocol was carried out in method B.

Method A



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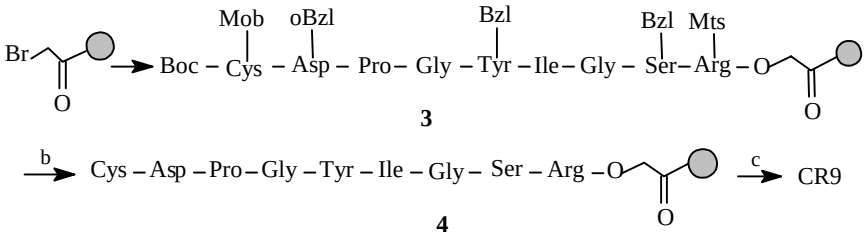
Method B



a) HF

b) Lo-TFMSA

c) NH_3 / MeOH EDT

Results and Discussion

There are always many by-products and impurities coexisting with targeted compound after HF cleavage in standard procedure like method A. So that the purification such as gel-filtration and/or preparative HPLC treatment must be followed. Because the OPac ester bond derived from Pac resin was very sensitive to nucleophilic attack and resistant to the condition for removal of side-chain protecting groups (SPGs), it was possible to remove SPGs from peptidyl-resin support without any premature cleavage of the OPac linker under Lo-TFMSA condition[5]. After this step, sufficient washes could drain off impurities such as reagents, scavengers and by-products, leaving only the naked-peptidyl resin.

The quite pure peptidyl amide could be released from the subsequent ammonolysis, and be collected very easily by trituration in ether after concentration. Because there was no further purification in method B, the yield of product was much higher than that in method A.

The differences between method A and B are summarized in **Table 1**.

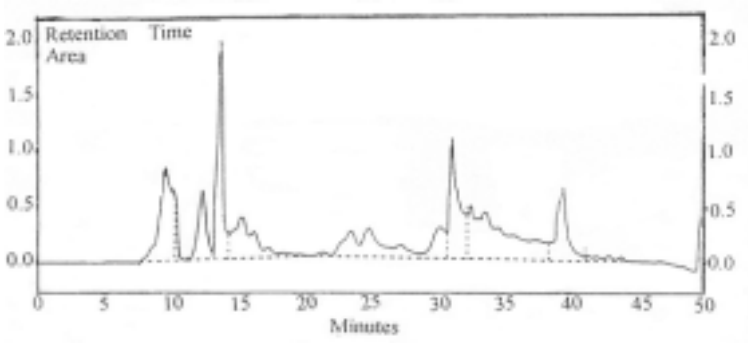
Table 1 Comparison of conditions between method A and method B

Conditions	method A	method B
Resin	MBHA~	Pac~
-Cys(x)-	Bzl-	*p*-MeO-Bzl-
-Arg(x)-	Tos	Mts
cleavage	HF	NH_3/MeOH
purification	Gel filtration,preparative-HPLC	ether trituation
lyophilization	yes	no
yields	12.4%	46.8%
ratio of cost	1	1/4

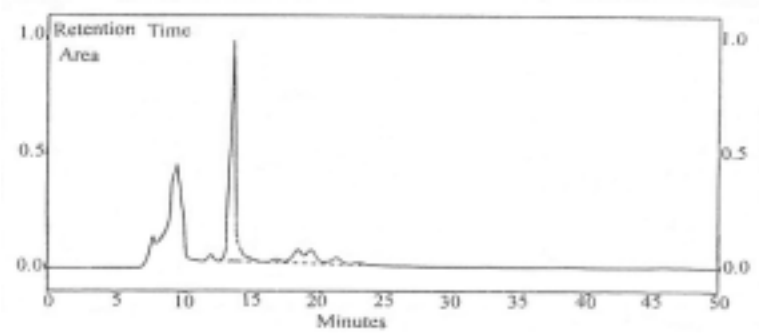
The purity of crude products from method A and method B was evaluated by HPLC analysis (**Figure 1**)[7]. It was obvious that the major peak around 14 minute in profile (a)、(b) and (c) should be the product fraction, and be confirmed by amino acid analysis and FAB-MS respectively. The other peaks with longer retention time appeared in

profile (a) should be responded to the additives from cleavage scavengers and by-products from side-chain protecting groups.

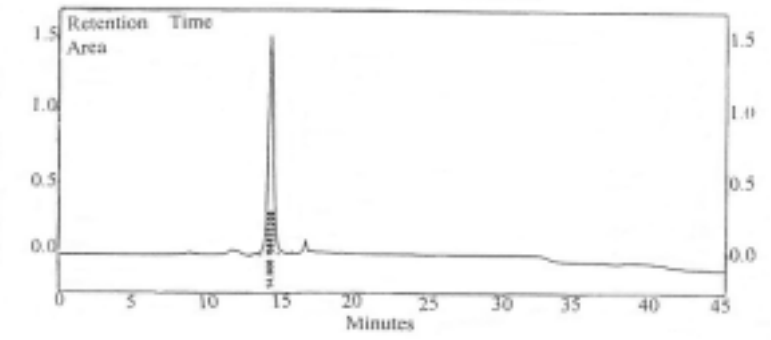
Figure 1 Profiles HPLC analysis of the products from method A and method B



(a) the crude product from method A



(b) the crude product from method B



(c) coinjection of two products after purification

The results indicated that an orthogonal strategy in stepwise deprotecting SPGs by acidolysis and releasing final product from solid support by amminolysis was a pragmatic way in the case of synthesis based on Pac resin. By this strategy, not only the product was released with convenient operation and in exempting from the special

aparatus for HF cleavage and in relatively high yield.

General Procedure

Method A

All coupling cycles and HF cleavage were carried out according to a typical procedure[6]. After lyophilization, 60 mg of CR9 was obtained. Based on 0.5 mmol MBHA resin the total yield was 12.4%. The product was confirmed by FAB-MS: 966.3 (M+1) and amino acid analysis: Asp (1) 1.05, Ser (1) 0.95, Gly (2) 2.05, Ile (1) 0.98, Tyr (1) 0.96, Cys (1) 0.94, Arg (1)1.00, Pro (1) 1.01.

Method B

The preparation of bromoacetyl resin and the attachment of Boc-Arg (Mts) onto this resin were carried out according to Mizoguchi method[4]. The followed procedure for assembly of nonapeotide on 0.3 mmol Boc-Arg (Mts)-OPac resin was the same as method A.

Whole SPGs were removed with Lo-TFMSA reagent[5]. The naked peptidyl-OPac resin was suspended in saturated ammonia methanol solution containing 1% EDT in a sealed tube for 24 h. Concentrating the supernatent and ether-trituating the residue gave 135 mg of white powder, with a yield of 46.8% based on the initial resin. This product was confirmed by FAB-MS: 966.1 (M+1) and amino acid analysis: Asp (1)1.03, Ser (1) 0.96, Gly (2) 2.07, Ile (1) 0.98, Tyr (1) 0.95, Cys (1) 0.95, Arg (1) 0.98, Pro (1) 1.00.

Acknowledgment

We thank our colleagues who work in MS lab. and amino acid analysis lab. for the identification of CR9.

References and Notes

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7. The conditons for HPLC analysis : Instrument type: Alltech 426, Column: Alltima C18,10u (10×250mm), Mobile phase: 31%CH_3CN/0.1%TFA, Wave length: 214 nm, Flow rate: 1mL/min.

Received 6 March, 2002