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# TEMPORARY $N^{\alpha}$-DEPROTECTION/REPROTECTION PROCEDURE TO FACILITATE THE PURIFICATION OF PROTECTED PEPTIDE FRAGMENTS FOR USE IN CONVERGENT SOLID PHASE PEPTIDE SYNTHESIS[1]

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***Abstract:*** *N*-Terminal Fmoc group of the fully protected peptide resin prepared by stepwise solid phase peptide synthesis in combination with $N^{\alpha}$-Fmoc protection, TFA-stable side-chain protections including newly developed 2-adamantyloxycarbonyl (2-Adoc) group, and TFA-cleavable solid support, was temporarily removed to give the polar intermediate, *profragment*, which was favorable for the purification by reversed phase mode rather than the *N*-terminal protected homolog. The purified profragment could be readily converted to the *N*-terminal protected fragment by treatment with Fmoc-OSu in short period for use in convergent solid phase peptide synthesis.

Convergent solid phase peptide synthesis, which involves the preparation of protected peptide fragments by stepwise solid phase peptide synthesis, followed by their purification and their assembly on the solid support, has been employed for the synthesis of some large peptides.[2] This strategy is particulary useful for overcoming the difficulties in the synthesis of large peptides, although some problems remain to be solved. One of the most critical problems in the convergent strategy, also common in solution phase peptide synthesis, is the unpredictable poor solubility of the protected peptide fragments, which makes their purification as well as their characterization and the subsequent fragment condensation difficult.

Recently, we reported the development of a new amino-protecting group, 2-adamantyloxycarbonyl (2-Adoc), and its application to the solid phase synthesis of protected peptide fragments corresponding to the sequences 25-35, 36-45, and 46-53 of metallothionein-like growth inhibitory factor (GIF).[3,4] The solubility of the protected fragments in organic solvent were actually improved by employing 2-Adoc. However, generalized method to further purify the protected peptides prepared by solid phase method have not been developed, and are now required in order to improve the convergent solid phase peptide synthesis.[2]

A possible strategy to facilitate the purification of the protected peptide fragments is achieved by introduction of the polar function into the molecule. Previously, it was reported that 3- and 4-picolyl protections of the side-chain functions of Ser, Thr, Asp and Glu could facilitate the purification of the protected peptide fragments.[5] The protected peptide fragments containing picolyl groups were actually more polar than the benzyl

homologs, and their purification by reversed phase MPLC was achieved successfully. However, the use of polar protecting groups would decrease the solubility in the organic solvent due to their hydrophilicity, while the solubility of the protected peptide fragments in water was actually enhanced. In addition, these protecting groups can not be removed by HF or 1M TFMSA-thioanisole/TFA, final deprotection and cleavage reagents in peptide synthesis, and the additional deprotection step, catalytic hydrogenation or electrolysis, is required. Recently, it was also reported that the temporary deprotection of $N^{\varepsilon}$-amino protecting group of Lys could facilitate the purification of the protected fragment because of enhanced solubility in water-containing solvent due to the polarity of $N^{\varepsilon}$-amino function.[6] As shown in Fig. 1a, the chain elongation in this strategy was depending on Boc-chemistry, and the photolytic cleavage was employed to retain $N^{\alpha}$-Boc and side-chain protective groups. The improvement of the solubility of the protected peptide in revresed phase medium due to its own polarity of the temporarily regenerated functional group is unique and efficient, although it is well known that photo-cleavage from Nbb resin is not quantitative, and gives highly contaminated product.[2,7] Here, we report the improved strategy to facilitate the purification the protected peptide intermediates, which involves 1) the removal of *N*-terminal Fmoc group following the stepwise solid phase synthesis of the protected peptide resin in combination with $N^{\alpha}$-Fmoc protection, TFA-stable side-chain protections and TFA-cleavable Wang resin, 2) cleavage from the resin by TFA to retain side-chain protections, 3) purification by reversed phase HPLC, and 4) reprotection of *N*-terminal amino function by Fmoc group (Fig. 1b). The efficiency of 2-adamantyl type protective groups in this strategy is also described.

To evaluate the above strategy, *N*-terminal Fmoc groups of the protected peptide resins **1a**, **2a** and **3a**, prepared previously,[4] were removed with 20 % piperidine in DMF. The resultant peptide resins were treated with

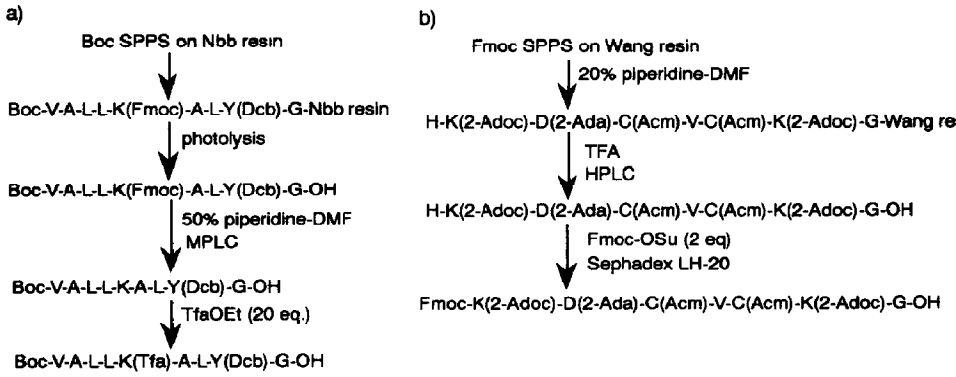


*Fig. 1 a) $N^{\varepsilon}$-deprotection/reprotection strategy and b) $N^{\alpha}$-deprotection/reprotection stratgy*

$R_1$-C(Acm)-K(2-Adoc)-C(Acm)-T(Bzl)-S(Bzl)-C(Acm)-K(2-Adoc)-S(Bzl)-C(Acm)-C(Acm)-$R_2$ **1**

$R_1$-S(Bzl)-C(Acm)-C(Acm)-P-A-E(O-Chp)-C(Acm)-E(O-Chp)-K(2-Adoc)-Cys(Acm)-$R_2$ **2**

$R_1$-A-K(2-Adoc)-D(O-2-Ada)-C(Acm)-V-C(Acm)-K(2-Adoc)-G-$R_2$ **3**

**1a**, **2a**, **3a**; $R_1$=Fmoc, $R_2$=Wang resin
**1b**, **2b**, **3b**; $R_1$=H, $R_2$=OH
**1c**, **2c**, **3c**, $R_1$=Fmoc, $R_2$=OH

*Fig. 2 Amino acid sequences and the side-chain protections of 1, 2 and 3*

TFA-phenol (95:5) to afford the side-chain protected peptides having the unprotected *N*-terminal amino function, **1b**, **2b** and **3b**. These intermediates seem just like to be the precursor of the fully protected peptide fragment, therefore, these are refered to "profragment". Profragments, **2b** and **3b**, were readily soluble in $H_2O$-MeCN, and efficiently purified by preparative HPLC using the standard $H_2O$-MeCN mobile phase system. The recoveries of them were >70 %, and the homogeneity of them were >95 % by analytical HPLC (Fig. 3b, and c). The profragment **1b**, containing three benzyl groups in 11 amino acid residues, was not soluble in $H_2O$-MeCN,

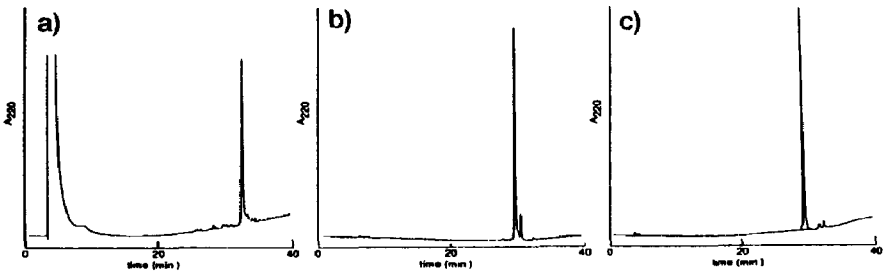


*Fig. 3 Analytical HPLC profiles of the purified profragments, (a) 1b, (b) 2b, and (c) 3b. Column: YMC-Pack R-C8-5, 4.6 x 250 mm (a), YMC-Pack Proteon-RP, 4.6 x 250 mm (b, c). Solvent system: A, $H_2O$ (0.05 % TFA); B, MeCN (0.05 %); A:B 80:20 for 5 min, 80:20 to 20:80 in 20 min, and 20:80 for 15 min.*

but soluble in $H_2O$-DMF. The recovery on the purification of **1b** was lower (ca. 20 %) than **2b** and **3b** presumably due to the low solubility of **1b** in the HPLC medium. It was readily predicted that the solubilty of the profragment would decrease in proportion to the increase of the number of hydrophobic moiety like benzyl, and use of less hydrophobic, but not polar protecting groups *i.e.* 2-Adoc for Lys, 2-Ada[8] for Asp, and Chp[9] for Glu were efficient to enhance the solubility of profragment in HPLC medium. Actually, the $H_2O$/MeCN soluble profragments, **2b** and **3b**, contained no or one benzyl moiety, respectively, while $H_2O$/MeCN insoluble profragment **1b** contained three benzyl groups. This result supports the effectiveness of our efforts to develop

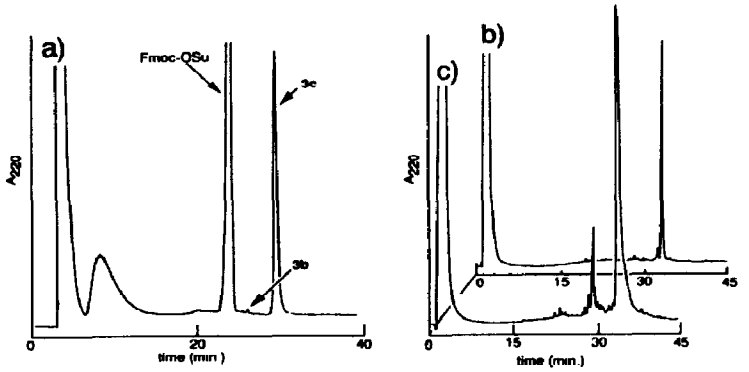


*Fig. 4 Convertion of profragment 3b to fragment 3c. a) reaction mixture (1 h) of 3b and Fmoc-OSu, b) 3c obtained via profragment, c) crude 3c obtained by direct cleavage of 3a. column: YMC-Pack Protein RP, 4.6 x 250 mm (a), Waters Nova-Pak C18, 3.9 x 150 mm (b, c). Solvent systems were same as those in Fig. 3.*

new side-chain protective groups alternative to benzyl type ones including 2-Ada and 2-Adoc. Subsequently, the purified profragments **1b**, **2b** and **3b**[10] were converted to the *N*-terminal protected fragments **1c**, **2c** and **3c**, by the treatment with Fmoc-OSu (2 eq.) in the presence of DIEA (2 eq.) in short period (<1 h, Fig. 4). The fragment **1c**, **2c** and **3c** were obtained in good yield (>80 %) and with a high degree of the purity (>95 % by analytical HPLC) after gel-filtration with Sephadex LH-20. The protected fragments obtained are soluble in DMF in suitable concentration for fragment condensation.

These results clearly demonstrated that profragment obtained by temporary *N*-terminal deprotection is favorable for the purification by reversed phase mode rather than the *N*-terminal Fmoc homolog in terms of improved solubility in HPLC medium. The conversion of profragment to fragment by the treatment with Fmoc-OSu in the presence of DIEA was completed in short period (<1 h), and the converted fragment was highly homogeneous without any purification. In addition, it is also expected that the profragment can be converted to the *N*-terminal Boc homolog, which can be used for further elongation depending on Boc-chemistry. In the profragment strategy, use of non benzyl-type TFA-stable protecting groups including 2-Adoc and 2-Ada was also efficient to facilitate the purification by HPLC, therefore, it was concluded that the combination of the profragment strategy with the use of 2-adamantyl-type protecting groups would be the most promissing approach to prepare the highly homogeneous protected peptide fragments for use in convergent solid phase peptide synthesis.

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**References and Notes**

1. *Abbreviations*: 2-Ada, 2-adamantyl; 2-Adoc, 2-adamantyloxycarbonyl; Boc, *t*-butyloxycarbonyl; Dcb, 2,6-dichlorobenzyl; DIEA, diisopropylethylamine; DMF, dimethylformamide; ESIMS, electro spray ionization mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; Fmoc-OSu, 9-fluorenylmethyl *N*-succinimidyl carbonate; HPLC, high performance liquid chromatograghy; MPLC, medium pressure liquid chromatography; Nbb, nitrobenzamidobenzyl; SPPS, solid phase peptide synthesis; Tfa, trifluoroacetyl, TFA, trifluoroacetic acid; TfaOEt, ethyl trifluoroacetate.
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