

# Fluoren-9-ylmethoxycarbonyl (Fmoc) Amino Acid Chloride as an Efficient Reagent for Anchoring Fmoc Amino Acid to 4-Alkoxybenzyl Alcohol Resin<sup>1)</sup>

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**Esterification of Fmoc amino acids to 4-alkoxybenzyl alcohol polystyrene through acid chlorides was achieved in high yield with practically no racemization. This reaction is especially effective for the rapid anchoring of amino acids which have bulky side chains.**

**Keywords** Fmoc amino acid chloride; anchoring; 4-alkoxybenzyl alcohol resin; racemization; substitution level; solid-phase peptide synthesis; Fmoc protection

Dicyclohexylcarbodiimide (DCC)–4-dimethylaminopyridine (DMAP)<sup>2)</sup> has been generally used for anchoring of the C-terminal amino acid onto 4-alkoxybenzyl alcohol resin<sup>3)</sup> (Wang resin). However, it has been shown that DMAP promotes racemization<sup>4)</sup> and dipeptide formation<sup>5)</sup> due to its basic character. To avoid these side reactions several modifications have been reported, but these have the disadvantage that only fairly low substitution levels can be obtained when a conventional polystyrene-derived support is used.<sup>6,7)</sup> The yield of esterification of the first amino acid to 4-alkoxybenzyl alcohol polystyrene resin is 80% or less even after a 15–20 h treatment with DCC–DMAP.<sup>7)</sup>

Various Fmoc amino acid chlorides have been synthesized and characterized by Carpino *et al.*<sup>8)</sup> Because of their high reactivity, these acid chlorides have been used for rapid continuous solution synthesis of peptides with practically no racemization.<sup>9)</sup> Fmoc amino acid chloride was also used for the acylation of a poor nucleophile, 5-bromo-7-nitroindoline, to give the acylation product in high optical purity.<sup>10)</sup> We now report the use of Fmoc amino acid chloride as an efficient anchoring reagent for 4-alkoxybenzyl alcohol polystyrene resin.

Fmoc amino acid chlorides were prepared according to the procedure described by Carpino *et al.*<sup>8)</sup> and used without purification. Each Fmoc amino acid chloride was dissolved in 40% pyridine in dichloromethane and the solution was vortexed with polystyrene-supported Wang resin at 25°C for 60 min. Pyridine, instead of DMAP, was used as a base to promote the coupling reaction since the Fmoc group is known to be completely stable to this weak base.<sup>11,12)</sup> The Fmoc group of each resulting resin was removed by treatment with 20% piperidine in DMF.<sup>12)</sup> The amount of each incorporated amino acid was determined quantita-

tively by using an amino acid analyzer after acid hydrolysis of the amino acid-resin. As shown in Table I, various amino acids, including sterically hindered Val, Ile, and Phe, were incorporated quantitatively onto the resin within 60 min. However, the extent of esterification of side-chain-protected His [His(Bom)<sup>13)</sup>] to the resin was fairly low (18%). In these coupling reactions, the choice of the reaction solvent is important. In dichloromethane, all amino acids except for His(Bom) were anchored to the resin quantitatively, whereas unsatisfactorily low coupling yields were obtained when DMF was used as the solvent. The low reactivity of Fmoc amino acid chlorides in DMA was also reported by Grandas *et al.*<sup>14)</sup>

The amount of D-isomer during each coupling was examined by the GITC (2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl isothiocyanate) procedure.<sup>15)</sup> Each amino acid-resin was treated with TFA at 25°C for 60 min and the cleaved amino acid was derivatized with GITC according to the procedure described by Kinoshita *et al.*<sup>16)</sup> The D-isomer content was determined quantitatively by reversed-phase high performance liquid chromatography (HPLC) (Table I). The D-isomer contents of amino acids except for Met were at or below the minimum detectable levels on HPLC, while a small amount (1.7%) of D-isomer was observed in the case of Met. It should be emphasized that practically no racemization (<0.5%) was observed in Phe or His residue, which is known to be very prone to racemization.<sup>7,17)</sup> These results agreed well with the literature data showing racemization-free acylations using several N<sup>α</sup>-protected amino acid (Phe, Ala, or Val) chlorides at 23–70°C for *ca.* 12 h.<sup>18)</sup>

In conclusion, Fmoc amino acid chloride was shown to be a simple, efficient, and racemization-free anchoring reagent for Fmoc-based solid-phase peptide synthesis. This acid chloride procedure is especially effective for the rapid and racemization-free anchoring of amino acids bearing a bulky side chain, although there are some limitations to the applicability of Fmoc amino acid chlorides bearing acid-sensitive side-chain protecting groups.

## Experimental

Thin layer chromatography (TLC) was performed on silica gel (Kiesel-gel 60F<sub>254</sub>, Merck) using CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (9:1:0.5) as a solvent system. Fmoc amino acids and 4-alkoxybenzyl alcohol polystyrene resin were purchased from Watanabe Chemical Industries, Ltd. Analytical HPLC was conducted with a Hitachi 655A. Amino acid analysis was conducted with a Hitachi L-8500.

**Fmoc Amino Acid Chlorides** Each acid chloride was prepared with

TABLE I. Yield and Racemization Amount in the Esterification of Fmoc Amino Acids to Wang Resin Using Fmoc Amino Acid Chlorides

	Yield (%)	D-Isomer (%)
Gly	≅ 100	—
Ala	≅ 100	0.7
Leu	96	<0.5
Val	≅ 100	<0.5
Ile	≅ 100	<0.5
Phe	≅ 100	<0.5
Met	≅ 100	1.7
His (Bom)	18	<0.5

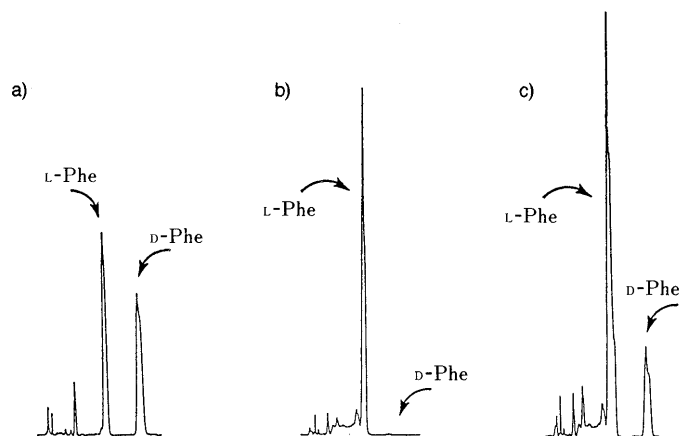


Fig. 1. Separation of Derivatized D-Phe and L-Phe Isomers on HPLC

a) Mixture of derivatized D-Phe and L-Phe isomers. b) Derivatized Phe-isomers formed from samples cleaved from H-Phe-Wang resin. c) Coelution of a) + b).

$\text{SOCl}_2$  in  $\text{CH}_2\text{Cl}_2$  according to the procedure described in the literature.<sup>8)</sup> The crude product obtained by the addition of *n*-hexane was used without further recrystallization. Each crude acid chloride contained ca. 10–40% of the starting Fmoc amino acid on TLC.

**Esterification to Wang Resin** 4-Alkoxybenzyl alcohol polystyrene resin (100 mg, 78  $\mu\text{mol}$ ) was placed in a polypropylene column (1.6  $\times$  8.0 cm) and Fmoc amino acid chloride (ca. 5 eq) in 40% pyridine in  $\text{CH}_2\text{Cl}_2$  (4 ml) was added. The mixture was vortexed at 25°C for 60 min. After washing of the resin with DMF, 20% piperidine in DMF (4 ml) was added and the mixture was vortexed at 25°C for 20 min. The resin was washed successively with DMF and  $\text{CH}_2\text{Cl}_2$ , dried, and then hydrolyzed in 12 N HCl–phenol–acetic acid (2:1:1, v/v)<sup>19)</sup> at 110°C for 20 h. The recoveries of amino acids are listed in Table I.

**Racemization Test** Amino acid-resin thus obtained (10 mg each) was treated with TFA (1 ml) at 25°C for 60 min. Dry ether (5 ml) and  $\text{H}_2\text{O}$  (2.5 ml) were added to the reaction mixture. The aqueous phase was separated from the organic phase, filtered, and then lyophilized. The product was dissolved in 50% (v/v) aqueous acetonitrile containing 0.4% (v/v)  $\text{Et}_3\text{N}$  (20  $\mu\text{l}$ ) and 2% (w/v) GITC in acetonitrile (20  $\mu\text{l}$ ) was added to the solution. The mixture was allowed to stand at 25°C for 30 min and an aliquot (4–20  $\mu\text{l}$ ) was injected directly into the chromatograph. The column (Cosmosil 5C18-ST, 4.6  $\times$  150 mm) was eluted at a flow rate of 0.9 ml/min with a mobile phase prepared by mixing methanol and 10 mM phosphate buffer (pH 2.8) in the following methanol ratios; 30% (His), 40% (Ala), 50% (Met), 55% (Val, Phe, Ile), and 60% (Leu). The eluate was monitored by UV absorption measurement at 250 nm. For the resolution of D-His isomer, the His(Bom)-resin (10 mg) was treated with 1 M  $\text{HBF}_4$ –thioanisole in TFA<sup>20)</sup> (1 ml) at 4°C for 60 min and the mixture was worked up as described above. A procedure described in the literature<sup>16)</sup> was employed for the preparation of the mixture of D- and

L-isomers. A typical resolution pattern on HPLC is shown in Fig. 1 and the D-isomer contents are summarized in Table I.

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#### References and Notes

- 1) Amino acids and derivatives mentioned in this investigation are of the L-configuration. The following abbreviations are used: Fmoc = fluoren-9-ylmethoxycarbonyl, Bom = benzyloxymethyl, TFA = trifluoroacetic acid, DMF = dimethylformamide, DMA = dimethylacetamide.
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