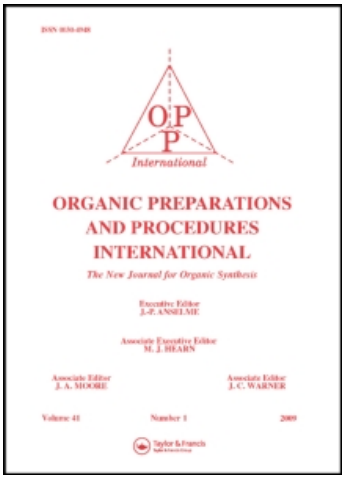

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AN EFFICIENT PROCEDURE FOR THE PREPARATION OF FMOC-AMINO ACIDS

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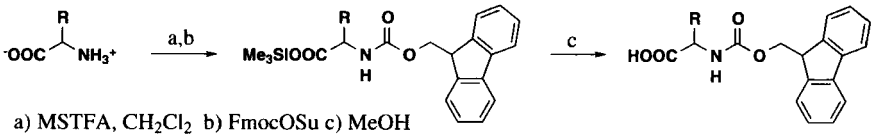
The 9-fluorenylmethoxycarbonyl (Fmoc) protecting group for amines was introduced in 1970 by Carpino and Han.[1] The early syntheses of Fmoc-amino acids made use of the highly activated Fmoc-Cl in a Schotten-Baumann type procedure.[1,2] It was later found that this procedure can lead to the formation of significant amounts of Fmoc oligopeptides which can be very difficult to remove.[3] Two methods have been developed that allow the synthesis of Fmoc-amino acids free of any corresponding Fmoc oligopeptides: one procedure uses Fmoc-N-succinimidyl carbonate (Fmoc-OSu) in mixtures of water and an organic solvent together with a base,[3,4,5] whereas the other consists of silylation of the amino acid with TMS-Cl and a tertiary amine base in an organic solvent followed by treatment with Fmoc-Cl.[6]

Concepts from each of the methods have been combined into a procedure that affords N^{α}-Fmoc-amino acids in high purity and yields under neutral, anhydrous conditions.



a) MSTFA, CH_2Cl_2 b) FmocOSu c) MeOH

An α-amino acid is suspended in methylene chloride and treated with two equivalents of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA). If the amino acid contains a hydroxyl group, such as in threonine or hydroxyproline, an additional equivalent of MSTFA is employed. The mixture is refluxed until a clear solution is obtained (1 to 8 hours depending upon the amino acid). Then one equivalent of Fmoc-OSu is added, and the resulting mixture is stirred at room temperature until the reaction is complete (2 to 73 hours depending upon the amino acid). The initial, silylated Fmoc-amino acid is converted to the free Fmoc-amino acid by treatment with methanol. Because the product mixture is neutral, extremely simple workups are possible. Evaporation of the product mixture to dryness often yields a solid which can easily be purified by washing first with a mixture of methanol and 10% citric acid followed by washes with water (workup A). In cases where an oil is formed after evaporation of the reaction mixture, a standard extractive workup can be employed (workup B).

TABLE. N^{α}-Fmoc-amino Acids by the MSTFA Procedure

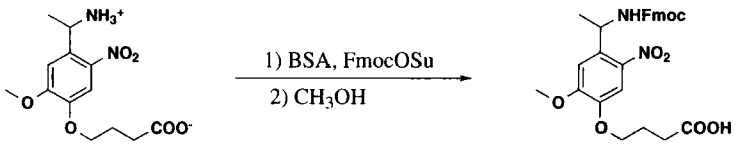
Compound	Time (h) Silylation	Fmoc	Workup	Yield (%)	mp. (°C)	lit. mp. (°C)
Fmoc-Gly	8	24	A	99[a]	171-175	173-175[6]
Fmoc-Ala	12	4.5	A	96[a,b]	145-147	141-142[6]
Fmoc-Phe	0.5	23	A	92[a,b]	184-187	183-184[6]
Fmoc-Pro	0.1	1.5	B	91[b]	106-109	96-99[6]
Fmoc-Met	1	72	A	90[b]	127-131	133-134[6]
Fmoc-Thr	2	70	B	91	165-166[c]	165-166[c,4]
Fmoc-(4-OH)-Pro	3	3.5	B	87	188-190[d]	122-124[9]
Fmoc-Lys (Boc)[e]	0.3	24	A	99	128-129	128-129[6]

a) Purity: <0.1% Fmoc-Oligomers by RP-HPLC.[7] b) Optical purity: < 1% racemization by chiral HPLC.[8] c) Measured as the dicyclohexylammonium salt. d) Identical to commercial sample (ChemImpex). e) Performed at room temperature.

The reaction times for the silylation reactions (see Table) are comparable to those reported for the TMS-Cl procedure.[6] The exceptions are glycine and alanine, which also were reported earlier to react only slowly in silylation reactions with acetamide-based silylating agents.[10] Due to the fact that the Fmoc-reactions are performed without the addition of an organic base, they tend to be somewhat slower than the ones in alternative protocols.[5,6] This can be explained by the liberation during the acylation reaction of N-hydroxysuccinimide which having a pK_a of 5.95,[11] leads to protonation of the α-amino group in the amino acid, a circumstance which reduces the rate of acylation. The silylation of N^{ε}-(Boc)-lysine was performed at room temperature because reaction at higher temperatures led to a transacylation which resulted in the formation of mixtures of N^{α}-Fmoc-Lysine-(Boc)-OH and N^{α}-Boc-Lysine-(Fmoc)-OH.

It is known that the addition of two equivalents of acetamide-based silylating agents to amino acids leads to the formation of the corresponding *bis*-silyl amino acids.[10] Our experiments with phenylalanine indicated that it is possible for the amino acid to be solubilized with only one equivalent of MSTFA in methylene chloride; however, the addition of Fmoc-OSu resulted in the precipitation of the free amino acid by the hydroxysuccinimide liberated in the reaction. Similarly it was found that 4-hydroxyproline could be solubilized with just two equivalents of MSTFA, but again the addition of Fmoc-OSu led to the precipitation of 4-hydroxyproline. To prevent any precipitation of amino acids, 2 equivalents of MSTFA should be employed in the reaction; in addition, one more equivalent of MSTFA should be used for each additional hydroxy group.

For large scale Fmoc-amino acid production, the less expensive N,O-*bis*(trimethylsilyl)-acetamide (BSA) may be employed. The following scheme illustrates its utility in the production of 4-{4-[1-(9-fluorenylmethoxycarbonylamino)ethyl]-2-methoxy-5-nitrophenoxy}butanoic acid, a useful photolinker reagent in combinatorial chemistry.[12]



1 2

As the experimental details indicate, the reaction conditions are the same as those for the α-amino acids. The workup, a simplified version of workup A, yields a product of 99.2% purity by HPLC.

EXPERIMENTAL SECTION

All melting points are uncorrected.

General Procedure for N^{α}-Fmoc-amino Acids.- The free amino acid (20 mmol) was suspended in 20 mL methylene chloride, and MSTFA (7.97 g, 40 mmol) was added. For amino acids with a free hydroxyl group, one more equivalent of MSTFA was added. The mixture was refluxed until a clear solution was obtained. This solution was then cooled to room temperature, and a solution of Fmoc-OSu (6.74 g, 20 mmol) in 30 mL methylene chloride was added over 10-15 minutes. The workup was initiated when Fmoc-OSu was completely consumed or when the amount of Fmoc-OSu did not decrease over a 2 hour period as indicated by TLC or HPLC. After the addition of 10 mL methanol the reaction mixture was stirred for 30 minutes and then evaporated to dryness with a rotary evaporator.

Workup A: If a solid formed after the evaporation, 30 mL of water was added and the mixture was stirred for 30 minutes. It was filtered and then washed three times with 10 mL 10% aqueous citric acid/methanol 1:1 followed by washes with water until the filtrate showed a neutral pH. The product was dried at 50° under vacuum.

Workup B: If an oil formed after the evaporation, the product was isolated via an extractive workup: Ether was added and the mixture was extracted three times with 5% potassium carbonate. The pH of the aqueous phase was then lowered to 2 with 1N HCl. If a solid precipitated it was collected by filtration, washed with water until the filtrate showed a neutral pH and then dried at 50° under vacuum. If an oil formed after adjusting the pH to 2, then the mixture was extracted three times with ethyl acetate. The organic phase was washed with water and brine and then dried over sodium sulfate. The solvent was removed by rotary evaporation, and the product was dried at 50° under vacuum. The HPLC purity of every N^{α}-Fmoc-amino acid described in this paper equaled or exceeded 99%.

Preparation of 4-{4-[1-(9-Fluorenylmethoxycarbonylamino)ethyl]-2-methoxy-5-nitrophenoxy}-butanoic Acid (2).- To a slurry of amino acid **1** (257.5 g, 0.86 mol) in methylene chloride (775 mL) was added N,O-*bis*(trimethylsilyl)acetamide (262 g, 1.3 mol). The mixture was refluxed for 75 min, whereupon the mixture became a solution. The heat source was removed and a solution of Fmoc-OSu (304.5 g, 0.9 mol) in methylene chloride (1300 mL) was added over a period of 20 minutes at 30°. After one hour, a heavy slurry had developed which was stirred at room temperature. After 16 hours, methanol (1800 mL) was added and stirring was continued for 5 hours. The mixture was

filtered (a slow filtration) and the solid was then reslurried four times with 1500 mL methanol each time. The product was dried at 65° and 50 mm Hg to yield 411.5 g (92%) photolinker reagent **2**, mp. 199-201°, lit.[12] mp. 200-201°.

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7. RP-HPLC: Machery Nagel 5-C8 column; Conditions: Gradient elution, 70% A (0.1% TFA in H_2O) and 30% B (0.1% TFA in CH_3CN) to 5% A in 20 minutes, 0.5mL/min.

8. Chiral HPLC: Phenomenex Chirex 3011 column; isocratic elution with 10mM ammonium acetate in methanol.

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