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Facile synthesis of Fmoc-*N*-methylated α- and β-amino acids

Thavendran Govender and Per I. Arvidsson*

Department of Biochemistry and Organic Chemistry, Uppsala University, Box 599, SE-751 24 Uppsala, Sweden

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Abstract—A highly efficient and environmentally friendly synthesis of Fmoc-*N*-methyl α- and β-amino acids from the corresponding Fmoc-amino acid, via intermediate oxazolidinones/oxazinanones, has been developed. Microwave heating for 3 min was required for the synthesis of the oxazinanones, while their Lewis acid catalyzed reductive opening only needed 1 min for completion. Hence, Fmoc-*N*-methyl-amino acids, suitable for, for example, solid phase peptide synthesis, can be readily prepared from the corresponding Fmoc amino acid in less than 1 h including purification. Fmoc-β^3-homophenyl alanine showed unanticipated reactivity, and provided a one-step route to the highly useful Fmoc-protected 1,2,3,4-tetrahydroisoquinolineaceticacid, that is a β-hTic analogue.

1. Introduction

N-Methylated α-amino acids are widespread in nature, as part of larger peptidic natural products. They also find broad application for the design of biologically active substances in medicinal chemistry.[1] Likewise, there have been many reports on synthesis and structural properties of biologically active substances containing β-amino acids, and on peptides composed of β-amino acids, that is β-peptides.[2] Peptides containing β^3-amino acids exclusively adopt a 14-helix, whereas peptides containing a mixture of α- and β-amino acids can adopt 11, 14 or 15-helix structures.[3] *N*-Methylated β-amino acids could be useful building blocks for further developments of foldamers, as well as in the synthesis of biologically active substances.[4] The use of Boc-protected *N*-methylated β-amino acids was previously reported by Seebach et al.[5] whereby sodium hydride and methyl iodide were employed for the synthesis. Herein we report the synthesis of Fmoc-*N*-methyl-β^3-amino acids through an alternative methodology.

Several procedures exist for the synthetic preparation of *N*-methylated amino acids;[6] one of the most useful being the preparation of *N*-protected *N*-methylated α-amino acids via reduction of 5-oxazolidines with triethylsilane and trifluoroacetic acid (TFA).[7]

We recently reported an improved methodology in which TFA could be replaced by a Lewis acid, for example, $AlCl_3$ (Scheme 1, $n = 0$).[8] This method was shown to be superior in terms of time, yield and protecting group tolerance; furthermore, it is better suited for large scale preparations. A recent report, showing that microwave irradiation could be used to synthesize the intermediate Fmoc-protected oxazolidines in just 2 min,[9] prompted us to investigate if microwave heating could enhance the whole reaction sequence, that is also the reductive opening of the oxazolidine. We were also interested to see if this overall reaction sequence could be applied for the preparation of Fmoc-protected *N*-methyl β-amino acids, a class of compounds previously not prepared according to this route (Scheme 1, $n = 1$).[10]

Fmoc-β^3-hAla was used to optimize reaction conditions. Conventional heating of the Fmoc amino acid **1a** with *p*-toluene sulfonic acid and paraformaldehyde in toluene led to mediocre yields in 2 h. Excess reagents and time were required to drive the reaction to completion. The yield and time were significantly improved with microwave heating for 3 min at 130 °C. In contrast to the previous report,[9] which used toluene as solvent, we opted for acetonitrile, as toluene has poor dielectric heating capacity. For the purpose of our investigation we used 1 mmol of Fmoc amino acids in all our experiments. However, the reaction was easily scaled up to 5 mmol of amino acid under the same reaction conditions. We also found it necessary to purify the product from unreacted amino acid and catalyst by filtration through a pad of silica before the reductive ring-opening, as

* Corresponding author. Tel.: +46 18 471 3787; fax: +46 18 471 3818; e-mail: Per.Arvidsson@kemi.uu.se

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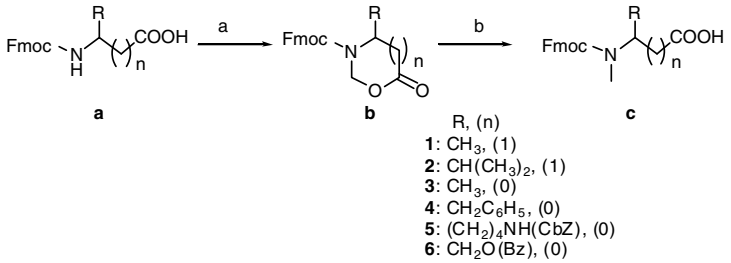


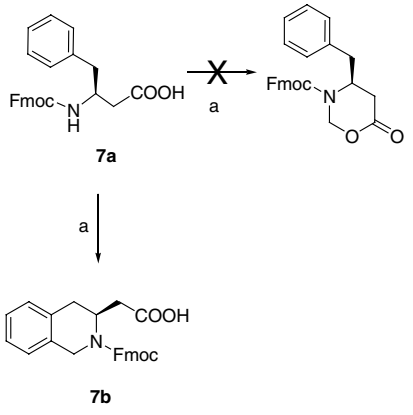
Scheme 1. Synthesis of *N*-methylated amino acids via reduction of 5-oxazolidines ($n = 0$)/oxazinanes ($n = 1$). Reagents: (a) $(CH_2O)_n$, *p*-toluene sulfonic acid (cat.), toluene, azeotropic removal of water; (b) Et_3SiH, $AlCl_3$, DCM.

separation of *N*-methylated- and starting Fmoc-amino acid is difficult.

Reduction of the intermediate 6-oxazinanone **1b** with $AlCl_3$ and triethylsilane in dry DCM afforded the Fmoc-*N*-methylated amino acid **1c** in 4 h at ambient temperature and only 1 min at 100 °C with microwave heating. This method can be employed for simple alkyl Fmoc-β^3-amino acids. The absence of racemization during this overall synthetic sequence was proven by synthesizing diastereomeric derivatives of (*R*)-α-methylbenzylamine with both enantiomers of Fmoc-*N*-methyl β^3-hAla. There was no sign of racemization when the resulting dipeptide was analyzed by NMR and reverse phase HPLC.

Attempts to synthesize *N*-methyl-Fmoc-β^3-hPhe failed with conventional and microwave heating. Instead of the expected product a surprisingly useful synthesis to 1,2,3,4-tetrahydroisoquinoline carboxylic acid derivative was realized (Scheme 2). Compounds of this type have gained interest due to their various biological activities and their ability to restrict the conformations of peptides.[11] Previous synthesis of similar compounds involved multiple steps with very poor overall yield.

We next decided to compare microwave heating (MH) and conventional heating (CH) on Fmoc-α-amino acids. In our previous report we obtained mediocre yields when synthesizing Fmoc-*N*-methyl-lysine with a Cbz side chain protecting group and therefore decided that this would be a good challenge. Unfortunately, we were unable to increase the yield of the Fmoc-Lys(Cbz) oxazolidinone formation (Table 1, entry 6). The oxazolidinone obtained from Fmoc-Ser(Bz), on the other hand could be prepared in good yields using both MH and CH (Table 1, entry 7). In most cases the yields were comparable for MH and CH (Table 1, entries 4–7), showing that no significant deprotection occurred under MH conditions.



Scheme 2. Attempted synthesis of Fmoc-β^3-hPhe resulted in an efficient synthesis of Fmoc-1,2,3,4-tetrahydroisoquinoline acetic acid. Reagents: (a) $(CH_2O)_n$, *p*-toluene sulfonic acid (cat.), heat.

Reduction of the oxazolidinone intermediate normally requires a reaction time of 4 h when using $AlCl_3$ and 24 h when using TFA at ambient temperature. We found that with microwave heating at 100 °C, the reaction was complete in just 1 min.

In conclusion, we have developed a facile microwave based methodology for the conversion of Fmoc-protected α- and β-amino acids into the corresponding Fmoc-protected-*N*-methyl amino acid. The overall procedure is highly efficient for non-side chain functionalized amino acid derivatives, and allows for medium size preparation (up to 5 mmol) of Fmoc-*N*-methyl-amino acids from the corresponding amino acid in less than 1 h. This smooth access to *N*-methyl amino acids advocates an even wider use of these derivatives in peptide synthesis and medicinal chemistry. Likewise, the efficient synthesis of Fmoc-1,2,3,4-tetrahydroisoquinoline acetic acid advocates a wider use of this interesting building block.

Infrared spectra were recorded on a Perkin–Elmer 1760 FT-IR spectrometer. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Unity 300 MHz spectrometer. High pressure liquid chromatography (HPLC) coupled to MS and universal evaporative light-scattering detection (ELSD) was done on a Gilson system consisting of a Gilson 322 pump, Gilson 233 XL autosampler and a Gilson UV/vis 152 detector, coupled in series with a Finnigan AQA mass spectrometer (electrospray in the positive mode) and an ELSD (Sedex 85 CC) from Sedere. The reverse phase HPLC analyses were done using a Phenomenex Gemini C18 (3 μm, 3.0 * 150 mm) column (for purity determination) with acetonitrile–water (both containing 0.1% formic acid) as mobile phase (gradient: 5–95% acetonitrile in 6 min+6 min at 95%, flow 1.0 ml/min) and for the diastereomers (gradient: 40–60% acetonitrile in 90 min). Microwave reactions were carried in sealed tubes in a Biotage Eight scientific microwave.

Table 1. Comparison between microwave and conventional heating for the synthesis of *N*-methylated Fmoc protected α- and β^3-amino acids

Entry	Fmoc amino acid	Conventional heating		Microwave heating		
		Time (h)	Yield (%) (product)	Conditions step 1	Yield (%)[a] (product)	Yield (%)[b] (product)
1	β^3-hAla (**1a**)	2	43 (**Ib**)	3 min @ 130 °C	82 (**1b**)	80 (**1c**)
2	β^3-hVal (**2a**)	2	43 (**2b**)	3 min @ 130 °C	96 (**2b**)	90 (**2c**)
3	β^3-hPhe (**7a**)	2	35 (**7b**)	3 min @ 130 °C	88 (**7b**)	—
4	Ala (**3a**)	1	97 (**3b**)	2 min @ 120 °C	97 (**3b**)	96 (**3c**)
5	Phe (**4a**)	1	92 (**4b**)	2 min @ 120 °C	92 (**4b**)	88 (**4c**)
6	Lys(Cbz) (**5a**)	1	55 (**5b**)	2 min @ 120 °C	52 (**5b**)	45 (**5c**)
7	Ser(Bz) (**6a**)	1	95 (**6b**)	2 min @ 120 °C	95 (**6b**)	10 (**6c**)

[a] Isolated yield after step 1.
[b] Isolated yields after the two step reactions.

2. General procedure for the synthesis of oxazolidinones/oxazinanones from Fmoc amino acids via conventional heating

The Fmoc amino acid (1 mmol), paraformaldehyde (200 mg) and *p*-toluene sulfonic acid (20 mg) were suspended in toluene (100 ml). The mixture was refluxed in a Dean–Stark setup until no more starting material could be detected by TLC (97.5:2:0.5 $CHCl_3$–MeOH–AcOH). The solution was cooled, washed with saturated $NaHCO_3$ and dried over anhydrous $MgSO_4$. Concentration in vacuo gave the crude product, which was filtered through a pad of silica gel by washing with ethyl acetate–pentane (3:7) until no more product was detected in the washing.

3. General procedure for the synthesis of oxazolidinones/oxazinanones from Fmoc amino acids via microwave heating

The Fmoc amino acid (1 mmol), paraformaldehyde (200 mg) and *p*-toluene sulfonic acid (20 mg) were suspended in acetonitrile (5 ml). The mixture was heated in a sealed microwave tube for the specified times. The solution was filtered through a pad of silica gel and washed with ethyl acetate–pentane (3:7) until no more product was detectable in the washing.

4. General procedure for the synthesis of *N*-methylated Fmoc amino acids from oxazolidinones/oxazinanones via microwave heating

To a solution of the oxazolidinone (1 mmol) and Lewis acid (2 mmol) in dry DCM (5 ml) was added triethylsilane (2 mmol) in a 5 ml microwave reaction tube. The reaction mixture was heated to 100 °C for 1 min. An additional amount of DCM (50 ml) was added and the organic phase was washed with 1 M HCl. The organic phase was dried over anhydrous magnesium sulfate and concentrated in vacuo. The crude product was purified via column chromatography on silica gel (97.5:2:0.5 $CHCl_3$–MeOH–AcOH).

The oxazolidinones and *N*-methyl amino acids of Fmoc-alanine, Fmoc-valine, Fmoc-phenylalanine, Fmoc-lysine(Cbz) and Fmoc-serine(Bz) were previously characterized.[6] The room temperature NMR spectra of the oxazinanes were broad and difficult to interpret. A study of the rotamers via low temperature NMR is not within the scope of this communication.

4.1. (*S*)-4-Methyl-6-oxo-[1,3]oxazinane-3-carboxylic acid 9*H*-fluoren-9-ylmethyl ester (1b)

Viscous oil; yield 82%; $[\alpha]_{589}^{25}$ +102.6 and $[\alpha]_{546}^{25}$ +122.3. IR (KBr): 1767, 1712, 1174 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 0.98–1.26 (br m, 3H); 2.42 (br m, 1H); 2.78 (br m, 1H); 4.23 (t, $J = 5.3$ Hz, 1H); 4.60 (br m, 2H); 5.05 (br m, 1H); 5.78 (m, 1H); 7.32 (t, $J = 7.4$ Hz, 2H); 7.42 (t, $J = 7.4$ Hz, 2H); 7.53 (t, $J = 7.4$ Hz, 2H); 7.80 (d, $J = 7.8$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 20.9 (q, br), 37.3 (t), 46.5 (d), 47.3 (d), 68.0 (t, br), 71.9 (t), 120.3 (d), 120.3 (d), 124.9 (d), 127.4 (d), 127.5 (d), 128.1 (d), 128.1 (d), 141.6 (s), 143.6 (s), 143.7 (s), 155.5 (s), 169.9 (s). MS (ESI) *m*/*z* 338.1 $[M+H]^+$.

4.2. (*R*)-4-Isopropyl-6-oxo-[1,3]oxazinane-3-carboxylic acid 9*H*-fluoren-9-ylmethyl ester (2b)

Amorphous solid; yield 96%; $[\alpha]_{589}^{25}$ +100.3 and $[\alpha]_{546}^{25}$ +120.0. IR (KBr): 1761, 1713, 1156 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 0.65–1.00 (br m, 7H); 2.44 (br m, 2H); 4.23 (t, $J = 5.3$ Hz, 1H); 4.43–4.92 (br m, 3H); 5.82 (m, 1H); 7.32 (t, $J = 7.5$ Hz, 2H); 7.42 (t, $J = 7.5$ Hz, 2H); 7.53 (t, $J = 7.5$ Hz, 2H); 7.80 (d, $J = 7.5$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 16.2 (q, br), 21.3 (d), 31.7 (t, br), 47.4 (d), 54.9 (d), 67.7 (t, br), 73.2 (t), 120.2 (d), 120.3 (d), 124.8 (d), 127.4 (d), 127.5 (d), 128.0 (d), 128.1 (d), 141.6 (s), 143.6 (s), 143.7 (s), 155.3 (s), 170.4 (s). MS (ESI) *m*/*z* 366.3 $[M+H]^+$.

4.3. (*S*)-3-[(9*H*-Fluoren-9-ylmethoxycarbonyl)-methyl-amino]-butyric acid (1c)

Waxy solid; yield 92%; $[\alpha]_{589}^{25}$ +21.0 and $[\alpha]_{546}^{25}$ +25.2. IR (KBr): 3064, 1728, 1700, 1147 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 0.96–1.30 (m, 3H); 2.22–2.78 (m, 2H); 2.79 (s, 3H); 4.08–4.70 (m, 4H), 7.20–7.42 (m, 4H); 7.59 (m, 2H); 7.75 (m, 2H); 9.60 (br s, 0.5H). ^{13}C NMR (75 MHz, $CDCl_3$): 18.0 (q), 29.1 (q), 39.1 (t), 47.5 (d), 49.2 (d), 67.6 (t), 120.1 (d), 125.2 (d), 127.2 (d), 127.8 (d), 141.5 (s), 144.2 (s), 156.3 (s), 176.6 (s). MS (ESI) *m*/*z* 340.1 $[M+H]^+$.

4.4. (*R*)-3-[(9*H*-Fluoren-9-ylmethoxycarbonyl)-isopropyl-amino]-butyric acid (2c)

Amorphous solid; yield 94%; $[\alpha]_{589}^{25}$ +0.35 and $[\alpha]_{546}^{25}$ +0.50. IR (KBr): 3167, 1731, 1697, 1147 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, rotamers): 0.60 (d, $J = 6.7$ Hz, 1.5H); 0.73 (d, $J = 5.9$ Hz, 1.5H); 0.86 (d, $J = 5.9$ Hz, 1.5H); 0.96 (d, $J = 6.7$ Hz, 1.5H); 1.39 and 1.86 (m, 1H); 2.09–2.38 (m, 1H); 2.50–2.70 (m, 1); 2.77 (s, 1.5H); 2.80 (s, 1.5H); 4.01–4.26 (m, 2H); 4.33–4.67 (m, 2H); 7.22–7.42 (m, 4H); 7.60 (m, 2H); 7.75 (m, 2H); 10.10 (br s, 0.5H). ^{13}C NMR (75 MHz, $CDCl_3$): 19.5 (q), 19.8 (q), 20.1 (d), 20.1 (d), 30.6 (q), 30.8 (q), 35.9 (t), 36.1 (t), 47.5 (d), 47.6 (d), 60.5 (d), 67.3 (t), 67.4 (t), 120.0 (d), 120.0 (d), 120.1 (d), 125.0 (d), 125.0 (d), 125.2 (d), 125.3 (d), 127.2 (d), 127.4 (d), 127.7 (d), 127.8 (d), 127.8 (d), 141.5 (s), 141.5 (s), 141.6 (s), 144.1 (s), 144.3 (s), 144.5 (s), 156.8 (s), 156.9 (s), 176.8 (s), 177.2 (s). MS (ESI) *m*/*z* 368.3 $[M+H]^+$.

4.5. (*S*)-3-Carboxymethyl-3,4-dihydro-1*H*-isoquinoline-2-carboxylic acid 9*H*-fluoren-9-ylmethyl ester (7b)

Amorphous solid; yield 88%; $[\alpha]_{589}^{25}$ +45.7 and $[\alpha]_{546}^{25}$ +54.7. IR (KBr): 3065, 1731, 1698, 1422, 1120, 740 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 2.35 (m, 2H); 2.80 (m, 1H); 3.08 (m, 1H); 4.17–5.05 (m, 5H); 7.05–8.90 (m, 13H). ^{13}C NMR (75 MHz, $CDCl_3$): 33.1 (t), 37.0 (t), 43.4 (t), 43.7 (t), 47.4 (d), 47.5 (d), 67.8 (t), 120.2 (d), 125.1 (d), 125.2 (d), 125.3 (d), 126.3 (d), 126.5 (d), 126.9 (d), 127.0 (d), 127.3 (d), 127.9 (d), 129.3 (d), 129.6 (d), 132.1 (s), 132.4 (s), 132.5 (s), 132.7 (s), 141.6 (s), 144.0 (s), 155.5 (s), 155.8 (s), 176.9 (s), 177.5 (s). MS (ESI) *m*/*z* 414.3 $[M+H]^+$.

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