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An improved synthesis of Fmoc-*N*-methyl serine and threonine☆

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Abstract—An improved method for the synthesis of Fmoc-*N*-methyl serine and threonine has been developed, which involves formation and subsequent reduction of the corresponding oxazolidinone with a Lewis acid under mild conditions, with improved yields and shorter reaction times.

1. Introduction

Structural conformations[1] and biological activity of synthetic peptides change drastically with the incorporation of N-methylated amino acids. Peptides containing N-methylated amide bonds often exhibit higher proteolytic stability,[2] increased membrane permeability,[3] enhanced duration of action and offer conformational rigidity.[4,5] A review by Fairlie et al. discusses many aspects of the biological activity of peptides including several examples of N-methylation in natural products and therapeutic agents.[6] N-Methylated peptides are generally synthesized by incorporation of Boc- or Fmoc-protected N-methylated amino acids, either by solution or solid phase peptide synthesis approaches.

Various methods have been developed for the synthesis of optically active *N*-methyl amino acids, both with functionalized and unfunctionalized side chains.[7,8] However, very few procedures exist for the synthesis of N-methylated β-hydroxy amino acids (Ser and Thr), which mainly give dehydrated derivatives, β-eliminated by-products or products which undergo partial racemization.[9–12] One of the mildest and most general procedures for the synthesis of *N*-methyl amino acids is based on the reduction of 5-oxazolidinones. The synthesis of oxazolidinones from N-protected amino acids and paraformaldehyde was originally devised by Ben-Ishai.[13] Subsequently, Freidinger et al. developed a method for reducing oxazolidinones with Et_3SiH and TFA.[14,15] Based upon this method, Luo et al. reported the preparation of *N*-Cbz and *N*-Fmoc *N*-methyl serine and threonine with TBDMS protection of the side-chain alcohol.[16] The (*O*-TBDMS) N-protected serine and threonine were reacted with paraformaldehyde and reduction of the corresponding oxazolidinone gave the desired *N*-methyl derivative with concomitant removal of the TBDMS protecting group. However, introduction of the TBDMS protecting group to Cbz serine or threonine required 2–3 days and further reduction of the oxazolidinones with Et_3SiH/TFA required 3–5 days stirring at rt. Also, this method resulted in a partial loss of the Fmoc protecting group, which had to be reintroduced.

Aurelio et al. reported the synthesis of *N*-benzyloxycarbonyl protected *N*-methyl serine and threonine along with other naturally occurring α-amino acids through 5-oxazolidinone reduction.[17] The β-hydroxyl group of the Ser or Thr side chain was protected by O-acetylation. Furthermore, reductive cleavage with TFA gave the benzyloxycarbonyl protected *N*-methyl serine/threonine. This method has been highly successful in generating novel *N*-benzyloxycarbonyl protected *N*-methyl amino acid derivatives. However, reductive cleavage with TFA again required a long reaction time.

As a result of these limitations, Zhang et al. developed a more efficient method for the reduction of the intermediate Fmoc-oxazolidinones of non-functionalized and

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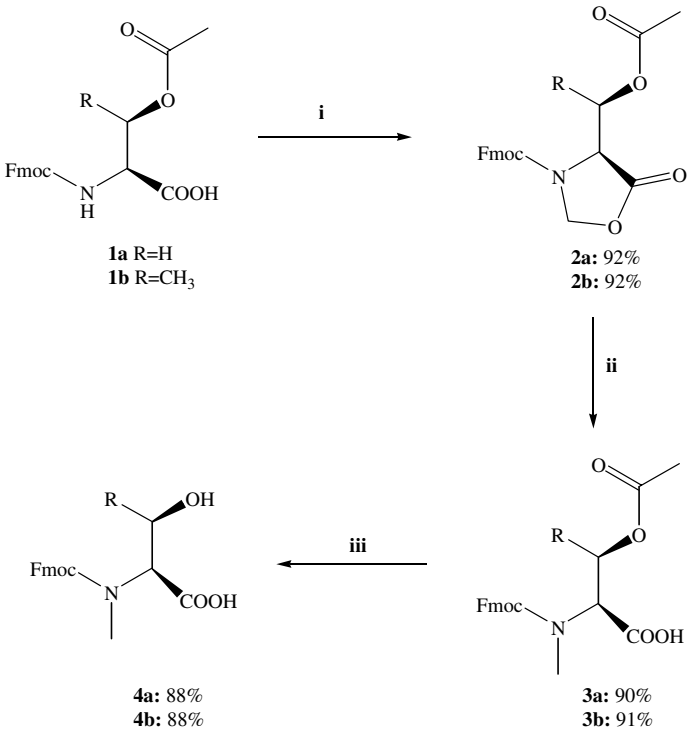

Scheme 1. Reagents and conditions: (i) paraformaldehyde, *p*-TsOH, toluene, 1 h reflux; (ii) $AlCl_3$, $(iPr)_3SiH$, DCM, 25 °C, 4 h; (iii) dioxane and HCl (2 M), 60 °C, 12 h heating.

functionalized side chain-containing amino acids, using a Lewis acid catalyst such as $AlCl_3$ or $ZnBr_2$.[18] Further, it was reported that the $AlCl_3$ reaction needed 4 h for completion whilst that with $ZnBr_2$ required 22 h, with negligible side product formation. However, when we attempted this reaction for the synthesis of Fmoc-*N*-methyl-Ser(OH)–OH and Fmoc-*N*-methyl-Thr(OH)–OH without side chain protection, no N-methylated product was obtained using either $AlCl_3$ or $ZnBr_2$. Chruma reported the synthesis of *N*-methyl amino acids, including Ser and Thr, by reductive amination of O'Donnell's Schiff base amino esters with $NaBH_3CN$ and the appropriate aldehyde in THF.[19] This method was found to be inadequate for the large scale production of N-methylated Ser and Thr, due to the difficulty in preparing the Schiff bases of the parent amino acids.

In the present Letter we report an efficient method for the synthesis of Fmoc-*N*-methyl serine and threonine. Among various functionalized amino acids, efforts were directed towards the synthesis of Fmoc-*N*-methyl serine and threonine because, in general, N-methylation of β-hydroxy amino acids is difficult, mainly due to β-elimination or dehydration and also, Fmoc-protected N-methylated β-hydroxyl amino acids can be easily utilized for solution or solid phase peptide synthesis.

As shown in Scheme 1, the *N*-Fmoc-protected *O*-acetyl-amino acids **1a**–**b**[20,21] were refluxed with paraformaldehyde and a catalytic amount of *p*-TsOH in anhydrous toluene to give the corresponding oxazolidinones **2a**–**b** in very good yields. Reduction of the oxazolidinone was achieved using $AlCl_3$ and triisopropylsilane ($(iPr)_3SiH$) giving Fmoc-*N*-methyl-*O*-acetyl amino acids **3a**–**b**. Finally, deprotection of the hydroxyl group was achieved using 2 M HCl giving Fmoc-N-methylated β-hydroxyl amino acids **4a**–**b** in very good yields.[22] Typically, the reaction time for the formation and subsequent reduction of the corresponding oxazolidinone was less than 1 h and the crude product obtained after work-up was sufficiently pure to be used in solid-phase peptide synthesis. Using this protocol, 500 g of both Ser and Thr Fmoc-protected N-methylated β-hydroxyl amino acids were prepared in good yields.

In conclusion, Fmoc N-methylated β-hydroxyl amino acids were prepared in excellent yields. This method allows large-scale preparation of Fmoc-protected N-methylated β-hydroxyl amino acids, with simple extraction yielding sufficiently pure compound. This process utilizes less expensive reagents and offers a shorter route for the synthesis of Fmoc-protected *N*-methyl-Ser and Thr amino acid derivatives.

2. General experimental procedures

Synthesis of oxazolidinone **2a**: Compound **1a** (100 g, 0.271 mol), paraformaldehyde (50.0 g) and *p*-TsOH (2.79 g, 0.06 equiv) were suspended in anhydrous toluene (5.5 L) and slowly heated to dissolve most of the starting material. The reaction mixture was then refluxed for 1 h using a Dean–Stark apparatus. The

reaction was cooled to rt and washed with 5% $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to yield **2a** as a colourless oil (94.9 g, 92% yield).[16] ^1H NMR ($CDCl_3$, 300 MHz): δ 7.76 (d, 2H, $J = 7.17$ Hz), 7.54 (d, 2H, $J = 7.31$ Hz), 7.39 (t, 2H, $J = 7.15$ Hz), 7.30 (t, 2H, $J = 7.33$ Hz), 5.22–5.10 (m, 2H), 4.83–4.81 (m, 1H), 4.49–4.42 (m, 2H), 4.24 (br s, 2H), 4.04–3.85 (m, 1H), 2.01 (s, 3H); IR (Nujol): 3068, 1714, 1421, 1355, 1109, 758 cm^{-1}; FABMS: m/z 382 $[M+1]^+$; Anal. Calcd for $C_{21}H_{19}NO_6$: C, 66.13; H, 5.02; N, 3.67%. Found: C, 66.21; H, 5.03; N, 3.69%.

Synthesis of Fmoc-N-methyl-O-acetyl amino acid **3a**: To a solution of Fmoc-protected oxazolidinone **2a** (90.0 g, 0.23 mol) and anhydrous $AlCl_3$ (62.8 g, 0.47 mol) in dry DCM (2.7 L) was added $(i\text{Pr})_3SiH$ (96.5 mL, 0.47 mol). The reaction mixture was stirred at ambient temperature until TLC showed the absence of starting material (~1 h). An additional amount of DCM (2.7 L) was added and the organic phase was washed with 1 M HCl (5.0 L). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford pure **3a** (81.4 g, 90% yield).[19] ^1H NMR ($CDCl_3$, 300 MHz): δ 9.63 (br s, 1H), 7.72 (d, 2H, $J = 7.21$ Hz), 7.51 (d, 2H, $J = 7.23$ Hz), 7.36 (t, 2H, $J = 7.24$ Hz), 7.29 (t, 2H, $J = 7.23$ Hz), 5.21–5.13 (m, 2H), 4.83–4.82 (m, 1H), 4.49–4.42 (m, 2H), 4.04–3.85 (m, 1H), 3.02 (s, 3H), 1.98 (s, 3H); IR (KBr): 2927, 1753, 1651, 1448, 1110, 761 cm^{-1}; FABMS: m/z 384 $[M+1]^+$; Anal. Calcd for $C_{21}H_{21}NO_6$: C, 65.79; H, 5.52; N, 3.65%. Found: C, 66.68; H, 5.53; N, 3.61%.

Synthesis of Fmoc-N-methyl amino acid **4a**: Compound **3a** (40.0 g, 0.10 mol) was suspended in a mixture of dioxane and 2 M HCl (800 mL, 1:1) with stirring. The mixture was then heated to 60 °C for 12 h. After cooling, the reaction mixture was diluted with water (4.0 L) and extracted with ether (3×800 mL). The combined organic phases were dried (Na_2SO_4), filtered and evaporated under reduced pressure to give **4a**, which was identical in all respects with previously reported material (31.3 g, 88% yield).[18] ^1H NMR ($CDCl_3$, 300 MHz): δ 9.67 (br s, 1H), 8.24 (br s, 1H), 7.71 (d, 2H, $J = 7.18$ Hz), 7.49 (d, 2H, $J = 7.21$ Hz), 7.38 (t, 2H, $J = 7.16$ Hz), 7.34 (t, 2H, $J = 7.24$ Hz), 4.51–4.48 (m, 3H), 4.46–4.42 (m, 2H), 4.04–3.85 (m, 1H), 3.07 (s, 3H); IR (KBr): 3630, 2820, 1749, 1657, 1452, 1046, 757 cm^{-1}; FABMS: m/z 342 $[M+1]^+$; Anal. Calcd for $C_{19}H_{19}NO_5$: C, 66.85; H, 5.61; N, 4.10%. Found: C, 66.81; H, 5.59; N, 4.08%.

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