New Strategies for an Efficient Removal of the 9-Fluorenylmethoxycarbonyl (Fmoc) Protecting Group in the Peptide Synthesis

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The aluminium trichloride/toluene system is investigated as a novel, unusual and straightforward reagent for the removal of the 9-fluorenylmethoxycarbonyl (Fmoc) protecting group in the solution peptide synthesis. This procedure avoids any undesired side reactions, such as the frequently observed inversion of the amino acid configuration.

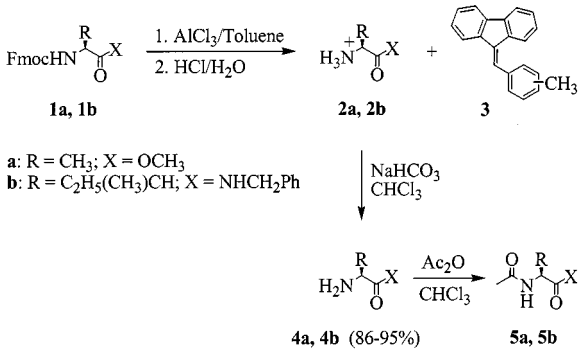
One of the most widely used strategies in the solid phase synthesis of peptides is based on the masking of the aminic function by the 9-fluorenylmethoxycarbonyl (Fmoc) group.[1] This approach has found extended applications due to the many advantages presented by the Fmoc protecting group, which can be easily removed under very mild basic conditions.[2] However, some limitations exist when this procedure is applied to the preparation of peptide fragments in solution.[3] In this case, in fact, a molar excess of nitrogenated base is required either for a complete removal of the Fmoc group from protected elongating peptide chains or for the quenching of dibenzofulvene which is generated during the unblocking step. Furthermore, it is necessary to remove the unreacted base from the reaction environment before performing the next coupling.[3,4] Finally, the pH controlled conditions adopted in the workup procedure do not allow an easy separation of the unprotected peptide from the reaction mixture containing the residual base and the dibenzofulvene-base adduct.

To overcome these problems, the use of alternative methods, such as catalytic hydrogenation,[5] has been evaluated to accomplish a successful removal of the Fmoc protecting group. Tetrabutylammonium fluoride (TBAF)[6] in the presence of thiols[7] has been found to be a suitable system for this purpose. The presence of sulfurated nucleophiles can ensure a rapid capture of dibenzofulvene and also minimises the major drawbacks associated with the purification of the reaction mixture containing different nitrogenated compounds.

The sensitivity of the base-labile Fmoc group to acid conditions has not been recognised before. This remarkable possibility could increase the potentiality of the protecting group either in the peptide synthesis or in the preparation of more complex base-sensitive organic substrates.

It seemed to be very attractive to exploit the Lewis acid assisted unblocking of the aminic function using new and straightforward strategies which could not employ heteroatom-containing reagents. The aluminium trichloride/toluene reacting system was thus tested as an alternative tool for the selective and highly efficient removal of the Fmoc group from both *N*-protected amino acids and dipeptide units.

The feasibility of the methodology was previously assessed using lipophilic amino acids as appropriate model compounds. In a typical experimental protocol, one mmol of the *N*-Fmoc protected derivatives **1a** or **1b** was treated with three mmol of $AlCl_3$ in dry toluene under an inert atmosphere (Scheme 1). The reactions went to completion in three hours at room temperature, as detected by TLC. After this time, the reaction mixture was acidified to give the hydrochloride of the corresponding amino acid derivative **2a** or **2b**. A simple solvent extraction with diethyl ether of the aqueous phase containing the unblocked compound afforded the substituted dibenzofulvenetoluene adduct **3** as a mixture of the *o*- and *p*-tolyl isomers. From these reactions, pure L-alanine methyl ester (**4a**) or L-isoleucine *N*-benzyl amide (**4b**) were obtained in good to excellent yields (86 and 95%, respectively). Each product was allowed to react with a molar excess of acetic anhydride in chloroform, at room temperature, to give **5a** and **5b**, whose structures were confirmed by GC-MS analysis performed in parallel with analytical samples of each *N*-derivatised compound.



Scheme 1. Removal of the Fmoc group by the $AlCl_3$/toluene reagent system

It could be supposed that, under the adopted conditions, the interaction between the Lewis acid $AlCl_3$ and the fulvene moiety affords a highly reactive electrophilic species which, in turn, forms a lipophilic adduct by reacting with

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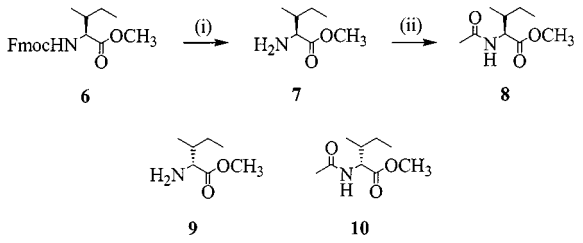
toluene. The advantages of this new methodology mainly consist in the use of toluene (the solvent of choice to carry out the unblocking step) as a suitable trapping agent for the fulvene derivative and the high lipophilicity of the dibenzofulvenetoluene adducts (which do not contain nitrogen) which can easily be separated from the other reaction system components. It is worth noting that the removal of the Fmoc group from the *N*-protected amino acid derivatives **1a** and **1b** occurred without any racemisation of the chiral α carbon atom. This aspect was extensively investigated using the *N*-Fmoc-L-isoleucine methyl ester (**6**), selected as the appropriate model to achieve our aim.

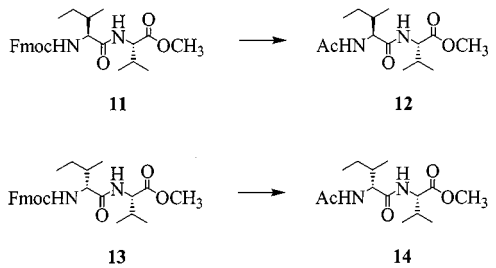
When **6** was treated with $AlCl_3$ in toluene, only the L-isoleucine methyl ester (**7**) was recovered in satisfactory overall yields (Scheme 2); no traces of the D-allo-isoleucine methyl ester (**9**) were detected. The absence of the inverted amino acid derivative **9** was confirmed by subjecting the crude material obtained from the treatment of **6** with $AlCl_3$ to acetylation. A GC-MS analysis of the resulting mixture did not reveal any *N*-acetyl-D-allo-isoleucine methyl ester (**10**), as established by injecting an authentic sample of compound **10**.



(i) 1. $AlCl_3$/Toluene; 2. HCl/H_2O; 3. $NaHCO_3/CHCl_3$ (ii) $Ac_2O/CHCl_3$

Scheme 2. The absence of racemisation was tested on the model compound *N*-Fmoc-L-Ile-OMe

We further report that any racemisation process was also suppressed in the unblocking of the *N*-Fmoc protected dipeptides (Scheme 3). As an example, *N*-Fmoc-L-Ile-L-Val-OMe (**11**) and *N*-Fmoc-D-allo-Ile-L-Val-OMe (**13**) were treated with $AlCl_3$ under experimental conditions similar to those adopted for the removal of the protecting group from **1a**, **1b** and **6**. The resulting mixtures were then acetylated in chloroform to afford the *N*-acetyl derivatives **12** and **14**, respectively, in very high yields. All the obtained compounds showed retention of configuration of the chiral carbon atom of the precursors, as confirmed by GC-MS and ^1H NMR spectroscopy.



Scheme 3. $AlCl_3$/toluene-induced unblocking of dipeptides: a proof of the complete retention of the amino acid stereochemistry

In conclusion we have been able to develop a new and convenient system for the removal of the Fmoc protecting group either from *N*-protected lipophilic amino acids or their ester or amide derivatives. We have also shown that an efficient trapping of the dibenzofulvene adduct, formed during the $AlCl_3$-promoted unblocking step, can be performed by the reaction solvent. The latter aspect is one of the most important features of the proposed procedure. This strategy can also be applied in the solution phase synthesis of dipeptides thus avoiding any unwanted inversion of the amino acid stereochemistry. Further applications of the optimised methodology to the removal of the Fmoc group from some side-chain functionalised amino acids, and to the preparation of longer peptide chains, is currently under investigation.

Experimental Section

General: Solvents and reagents were purified and dried by standard procedures and distilled prior to use. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were obtained with a Perkin–Elmer FT Paragon 1000 PC spectrometer. ^1H NMR spectra were recorded at 300 MHz on a Bruker ACP 300 spectrometer with tetramethylsilane as internal standard and $CDCl_3$ or [D_6]DMSO as solvents. Chemical shifts (δ) are given in ppm. GC/MS analyses were carried out on a Hewlett-Packard HP 5890 Series II gas chromatograph coupled to an HP 5972 A Series mass-selective detector with a 30 m HP-5MS capillary column with a 0.25 mm internal diameter and a 0.25 μm film thickness (Hewlett-Packard). The mass detector was operated in the electron-impact ionisation mode (EIMS) with an electron energy of 70 eV. Mass spectra were recorded on a Fisons Vacuum Generators ZAB-2F spectrometer, from 2 mL of 3-nitrobenzyl alcohol (NBA)/sample mulls, by fast atom bombardment (FAB^+MS), with a neutral xenon beam operating at 8 keV and a total current of 10 μA. Reaction mixtures were monitored by TLC using Merck Silica Gel 60-F_{254} precoated glass plates. When required, the reactions were carried out under inert atmosphere (N_2).

Synthesis of *N*-acetyl-l-Ile-NHCH$_2$Ph (5b): $AlCl_3$ (0.40 g, 3 mmol) was added to a magnetically stirred solution of *N*-Fmoc-L-Ile-$NHCH_2Ph$ (**1b**) (0.44 g, 1 mmol) in dry toluene (20 mL). The resulting mixture was maintained at room temperature for 3 h, monitoring the conversion of the protected precursor by TLC ($CHCl_3$/CH_3OH, 90:10). HCl 1N was then added and the acidified solution (pH = 2) was extracted with diethyl ether (3 × 10 mL). The aqueous phase was made basic with a saturated $NaHCO_3$ solution, then extracted with chloroform (4 × 10 mL) and washed with distilled water (2 × 10 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness to give pure **4b** (0.21 g, 95% yield). A solution of the latter compound (0.21 g, 0.95 mmol) in chloroform (8 mL) was allowed to react with acetic anhydride (0.18 mL, 1.9 mmol) at room temperature for 20 min. The mixture was made basic with a saturated $NaHCO_3$ solution, then extracted with chloroform (3 × 6 mL). The organic phase was washed with distilled water (2 × 6 mL), then dried (Na_2SO_4) and evaporated to dryness to afford **5b** (0.17 g, 92% yield) as a white solid, m.p. 51–53 °C. – IR (KBr): nu(tilde) = 3286, 3088, 2964, 1632, 1540, 1380, 730, 696 cm^{-1}. – ^1H NMR ([D_6]DMSO): δ = 0.75–0.90 (m, 6 H, C*H*$_3$CH and C*H*$_3$CH$_2$), 1.10 (m, 1 H, CH$_3$C*H*$_2$), 1.42 (m, 1 H, CH$_3$C*H*$_2$), 1.72 (m, 1 H, CH$_3$C*H*), 1.89 (s, 3 H, CH_3CO), 4.22 (m, 1 H,

NC*H*CONHCH$_2$Ph), 4.28 (d, J = 5.9 Hz, 2 H, HNCH_2), 7.22–7.38 (m, 5 H, Ar-H), 7.98 (d, J = 8.7 Hz, 1 H, CH$_3$CON*H*CH), 8.53 (t, J = 5.9 Hz, 1 H, *H*NCH$_2$). – GC-MS (EI); *m/z* (%): 262 (1) [M$^+$], 206 (6), 156 (11), 128 (57), 106 (25), 91 (51), 86 (100), 43 (32). – FAB$^+$-MS (+, NBA); *m/z* (%): 263 (86) [(M + H)$^+$], 128 (100).

Synthesis of Dipeptides 11 and 13: L-Val-OMe hydrochloride (0.32 g, 1.91 mmol) dissolved in a 5% aqueous Na_2CO_3 solution (13 mL) was added dropwise to the appropriate *N*-Fmoc amino acid chloride (1.91 mmol) in ethanol-free chloroform (13 mL). The resulting mixture was maintained under vigorous magnetic stirring at room temperature for 40 min, until complete conversion of the methyl ester (TLC: $CHCl_3$/CH_3OH 90:10). The aqueous phase was separated, then extracted with chloroform (3 × 10 mL). The collected organic layers were washed with HCl 0.1N (2 × 5 mL), distilled water (1 × 5 mL) and evaporated to dryness to afford the corresponding dipeptide (88–96% overall yields).

11: White solid, m.p. 163–165 °C. – IR (KBr): nu(tilde) = 3293, 2964, 1736, 1691, 1650, 1533, 1242, 1125, 730, 668 cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 0.80–0.97 [m, 12 H, (CH_3)$_2$CH, CH_3CH and CH_3CH$_2$], 1.17 (m, 1 H, CH$_3$CH_2), 1.53 (m, 1 H, CH$_3$CH_2), 1.84 (m, 1 H, CH$_3$C*H*), 2.15 [m, 1 H, (CH$_3$)$_2$C*H*], 3.71 (s, 3 H, OCH$_3$), 4.11 (m, 1 H, NCHCO),), 4.21 (m, 1 H, C*H*CH$_2$O), 4.39 (m, 2 H, CHCH_2O), 4.54 (dd, J_1 = 5.0 Hz, J_2 = 8.6 Hz, 1 H, NC*H*COOCH$_3$), 5.56 (d, J = 8.7 Hz, 1 H, NH Ile), 6.51 (d, J = 8.6 Hz, 1 H, NH Val), 7.25–7.78 (m, 8 H, Ar-H). – FAB$^+$-MS (+, NBA); *m/z* (%): 467 (100) [(M + H)$^+$], 435 (2), 336 (2.3), 271 (7), 245 (15), 165 (43).

13: White solid, m.p. 184–186 °C. – IR (KBr): nu(tilde) = 3294, 2926, 1734, 1687, 1648, 1541, 1262, 1094, 756, 738 cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 0.78–0.89 [m, 12 H, (CH_3)$_2$CH, CH_3CH and CH_3CH$_2$], 1.14 (m, 1 H, CH$_3$CH_2), 1.36 (m, 1 H, CH$_3$CH_2), 1.88 (m, 1 H, CH$_3$C*H*), 2.09 [m, 1 H, (CH$_3$)$_2$C*H*], 3.64 (s, 3 H, OCH$_3$), 4.12–4.23 (m, 2 H, NCHCO and C*H*CH$_2$O), 4.34 (d, J = 6.8 Hz, 2 H, CHCH_2O), 4.49 (dd, J_1 = 4.8 Hz, J_2 = 8.3 Hz, 1 H, NC*H*COOCH$_3$), 5.33 (d, J = 8.0 Hz, 1 H, NH Allo-Ile), 6.39 (d, J = 8.3 Hz, 1 H, NH Val), 7.18–7.75 (m, 8 H, Ar-H). – FAB$^+$-MS (+, NBA); *m/z* (%): 467 (100) [(M + H)$^+$], 435 (1.6), 336 (2.4), 271 (14), 245 (34), 165 (68).

Synthesis of Dipeptides 12 and 14: The title compounds were obtained by adopting a procedure similar to that previously described for the preparation of **5b**.

12: White solid, m.p. 158–161 °C. – IR (KBr): nu(tilde) = 3289, 2963, 1733, 1634, 1557, 1262, 1104, 800 cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 0.80–0.90 [m, 12 H, (CH_3)$_2$CH, CH_3CH and CH_3CH$_2$], 1.09 (m, 1 H, CH$_3$CH_2), 1.49 (m, 1 H, CH$_3$CH_2), 1.73 (m, 1 H, CH$_3$C*H*), 1.94 (s, 3 H, CH$_3$CO), 2.10 [m, 1 H, (CH$_3$)$_2$C*H*], 3.67 (s, 3 H, OCH$_3$), 4.33–4.43 (m, 2 H NCHCO and NC*H*COOCH$_3$), 6.50 (d, J = 8.5 Hz, 1 H, NH Ile), 6.78 (d, J = 8.3 Hz, 1 H, NH Val). – GC MS (EI), *m/z* (%): 230 (11), 128 (100), 100 (18), 86 (90), 72 (43), 43 (33). – FAB$^+$-MS (+, NBA); *m/z* (%): 287 (100) [(M + H)$^+$].

14: White solid, m.p. 167–170 °C. – IR (KBr): nu(tilde) = 3289, 2963, 1733, 1634, 1557, 1262, 1021, 800 cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 0.83–0.88 [m, 12 H, (CH_3)$_2$CH, CH_3CH and CH_3CH$_2$], 1.10 (m, 1 H, CH$_3$CH_2), 1.43 (m, 1 H, CH$_3$CH_2), 1.83 (m, 1 H, CH$_3$C*H*), 1.95 (s, 3 H, CH$_3$CO), 2.10 [m, 1 H, (CH$_3$)$_2$C*H*], 3.65 (s, 3 H, OCH$_3$), 4.40–4.55 (m, 2 H, NCHCO and NC*H*COOCH$_3$), 6.30 (d, J = 8.8 Hz, 1 H, NH Allo-Ile), 6.65 (d, J = 8.5 Hz, 1 H, NH Val). – GC MS (EI); *m/z* (%): 230 (14), 128 (100), 100 (15), 86 (80), 72 (33), 43 (40). – FAB$^+$-MS (+, NBA); *m/z* (%): 287 (100) [(M + H)$^+$].

Acknowledgments

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