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'One-Pot' Methylation of Fmoc Amino Acids[1]

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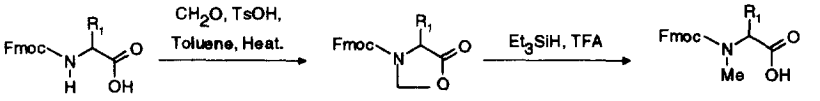
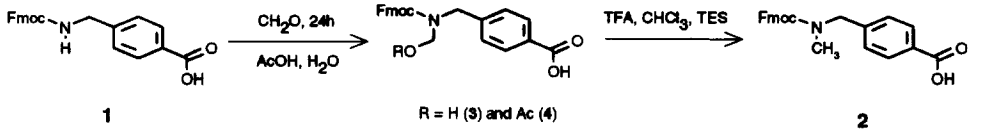
Abstract: *We report here a convenient method for the conversion of Fmoc amino acids to their N-methylated derivatives. The method involves reaction with formaldehyde and then reduction with triethylsilane (TES). The method is particularly attractive in that the alkylation and reduction are carried out* in situ *and the reaction sequence and purification of product can readily be carried out in one day. A further advantage is that the method is not restricted to α-amino acids.*

Many laboratories have large collections of *N*-Fmoc[2] protected amino acids that are used to synthesise peptides as part of structure activity studies. Due to the often remarkably different biological activity of *N*-methylated peptides it is useful to be able to convert these *N*-Fmoc protected amino acids to the corresponding *N*-methyl-*N*-Fmoc compounds when required. Freidinger *et al* developed a very useful two step method for this conversion for α-amino acids[3] (scheme 1) but to the best of our knowledge a 'one-pot' method applicable to all amino acids has not been reported to date.



Scheme 1

We recently wished to convert the Fmoc derivative of *para*-aminomethylbenzoic acid (**1**) to its N-methyl derivative (**2**) but as expected the Freidinger method was unsuitable for this non-α amino acid. However we found that treatment of the Fmoc amino acid (**1**) with formaldehyde in acetic acid at room temperature led to formation of a mixture of the methylol (**3**) and methylacetate (**4**) derivatives (on one occasion both were isolated by preparative HPLC and the identities confirmed by ^{1}H NMR and MS). Reduction of this mixture with triethylsilane (TES) in trifluoroacetic acid (TFA) gave the desired N-methyl derivative (**2**)[4] in 59% overall yield (scheme 2).[5]

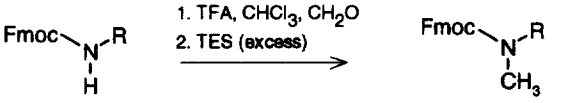


1 R = H (3) and Ac (4) 2

Scheme 2

The method also worked well for the Fmoc derivative of *meta*-aminomethylbenzoic acid giving 60% yield of the *N*-methyl analogue. However, we found that this method was somewhat capricious, especially when applied to other amino acids, and often gave poor yields. The reaction with formaldehyde usually appeared to go to completion (from RP-HPLC[2] evidence) but work up often led to reversion to starting materials. This instability of the *N*-methylol urethanes is not unexpected as the equilibrium often lies on the side of the starting materials and requires an excess of formaldehyde to drive the reaction to completion.[6] Due to these problems we attempted to improve the method.

The simplest way to avoid the problem of reversion to starting materials appeared to be to carry out the reduction *in situ*, thereby avoiding the work up altogether. We found that the methylol derivatives could be formed in 50:50 TFA:chloroform in less than 30 minutes and that addition of excess triethylsilane (TES) led to formation of the *N*-methyl derivatives within minutes. An excess of TES is necessary, presumably because under these conditions it also reduces formaldehyde to methanol. The reaction mixture was then concentrated under vacuum and the product purified by silica gel chromatography. One of the advantages of this second method is that the entire procedure of reaction and purification can easily be carried out within one day (Scheme 3).[7]



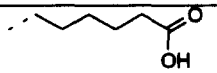
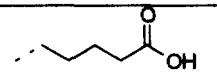
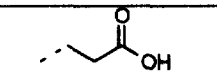
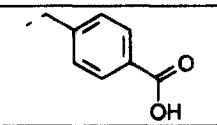
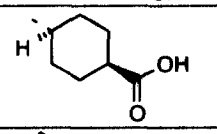
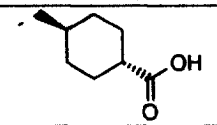
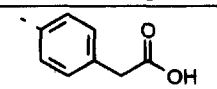
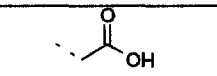
Scheme 3

A variety of amino acids were investigated (Table 1).

In general good to excellent yields were obtained but poor yields were found where the amino group and the carboxylic acid were closest together (**7** and **11**) or the amino group was less reactive (**10**).

Because of the existence of the Freidinger method for the methylation of Fmoc-α-amino acids we have not extensively examined the utility of our method for these compounds. However, it would appear from a limited number of examples (data not shown) that it is suitable although the yields are disappointing.

Table 1. 'One-Pot' Methylation of Fmoc Amino Acids.

Compound No	Fmoc-N(CH_3)-R ; R =	FORM	YIELD %	PURITY[a] %
5		oil	100	>95
6		oil	68	>95
7		crystalline	43	76
2		crystalline	94	>95
8		oil	100	>95
9		crystalline	91	>95
10		crystalline	51	92
11		crystalline	27	93

Note a: Purity determined by RP-HPLC (monitoring at 230nm).

In summary, we have described a convenient method for the conversion of Fmoc amino acids to their *N*-methylated derivatives. The method involves reaction of the NH compound with formaldehyde and then reduction with triethylsilane (TES). The method is particularly attractive in that the alkylation and reduction are carried out *in situ* and the process can readily be carried out in one day. Furthermore, the method is not restricted to α-amino acids.

REFERENCES AND NOTES

1. Initial results were presented at the First Australian Peptide Conference, Queensland, 16-21st October 1994.

2. Abbreviations: Fmoc, fluorenylmethoxycarbonyl; TFA, trifluoroacetic acid; TES, triethylsilane; RP-HPLC, Reverse Phase - High Performance Liquid Chromatography.

3. R.M. Freidinger, J.S. Hinkle, D.S. Perlow, and B.H. Arison, *J. Org. Chem.* **1983**, *48*, 77-81.

4. All new compounds gave the expected proton NMR spectra, mass spectra and either accurate mass or microanalysis.

5. Two stage synthetic procedure to Fmoc-N(Me)-$CH_2pC_6H_4CO_2H$ (**2**): *Fmoc-$NHCH_2pC_6H_4CO_2H$ (1.92g, 5.15 mmol) was dissolved in glacial acetic acid (270 ml) and 40% aqueous formaldehyde (20 ml, 270 mmol, a 13:1 ratio of solvent to formalin, use of a lower concentration of formaldehyde resulted in incomplete reaction) was added. The solution was left to stand for 24h then poured into water (1000 ml) and the resulting precipitate extracted three times with ethyl acetate (3 x 250 ml). The organic layer was washed with water, then saturated brine, then dried over anhydrous sodium sulphate and solvent removed under reduced pressure. The resulting white emulsion was dissolved in chloroform (30 ml) and trifluoroacetic acid (30 ml) and treated with triethylsilane (2.5 ml, 15.8 mmol, 3eq). The solution was stirred for 90 minutes at room temperature, then the solvents were evaporated off, and the residue co-evaporated three times with chloroform (3 x 100ml). The oil formed was triturated with ether giving 1.22g (59%) of product (2) as a colourless solid: mp 177-178° ; NMR (DMSO) δ 2.75 (2s, 3, N-CH_3), 4.2-4.5 (m, 5, -CH-CH_2-O, -CH-CH_2-O-, -N-CH_2-), 7.0-8.0 (m, 12, Ar**H**), 12.85 (br s, 1, CO_2**H**), some doubling of peaks was seen, presumably due to rotational isomers about the urethane bond; mass spectrum, m/e (FAB-) 386 $(M-H)^-$; found, C, 74.2, H, 5.42, N, 3.5, theory, C, 74.4, H, 5.46, N, 3.62.*

6. H.E. Zaugg and W.B. Martin, *Org. React.* **1965**, *14*, 52.

7. 'One-Pot' synthetic procedure to Fmoc-N(Me)-$CH_2pC_6H_4CO_2H$ (**2**): *Fmoc-$NHCH_2pC_6H_4CO_2H$ (0.509g, 1.36 mmol) was dissolved in TFA (7.5 ml) and $CHCl_3$ (7.5 ml) and the solution was treated with 40% aqueous formaldehyde (1.2 ml, 16 mmol, a ratio of 13:1 solvent to formalin). After 30 minutes, TES was added (3.17 ml, 20 mmol). The reaction was followed by RP-HPLC, and after 30 minutes was complete. The solvents were evaporated off and the residue co-evaporated three times with $CHCl_3$. The product was purified by silica gel chromatography, eluting with ethyl acetate. The oil obtained was triturated with isohexane to yield the product (2) 499 mg (94%) as a colourless solid (analysis as above[5]).*

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