# Efficient Acylation of Hydroxy Functions by Means of Fmoc Amino Acid Fluorides

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Fmoc amino acid fluorides are ideally suited for the efficient esterification of hydroxy resins to give high loading levels with low racemisation.

The efficient acylation of hydroxy-functionalised solid supports with Fmoc-protected amino acids without racemisation is the essential first step for a successful solid phase synthesis (SPPS) of peptide acids. In the early days of SPPS such reactions were carried out using preformed symmetrical anhydrides [*via* dicyclohexylcarbodiimide (DCC)] in the presence of 4-(dimethylamino)pyridine (DMAP).[1–4] However, this method was subsequently shown to be problematic due to difficulties arising from the high basicity of DMAP. Side reactions leading to extensive racemisation[5] and premature Fmoc-deblocking with consequent dipeptide formation[4] were reported. Fmoc-protected amino acid fluorides are stable in the presence of tertiary amines[6] although a slow conversion to the oxazolone was found in the case of the more sensitive α,α-disubstitued amino acids in the presence of bases such as pyridine, collidine, *N*-methylimidazole (NMI), *N*-methylmorpholine (NMM), DIEA or DMAP. These findings prompted a systematic investigation of the acid fluoride method for the acylation of hydroxy-functionalised resins. Previous studies were limited to a few amino acids or involved conditions which were not optimised for minimum racemisation levels.[7,8]

Initial model studies were carried out on the acylation of benzyl alcohol by means of Boc-Ala-F in the presence of various bases† (Table 1). Rough studies of the acylation rate demonstrated the exceptional acceleration by DMAP[9] ($t_{1/2}$ < 2 min). The efficiency of other bases is influenced by both their basicity (possible conversion of the hydroxy group to the corresponding alkoxide[10]) and the effect of steric hindrance around the nitrogen atom (possible intermediate conversion of the acid fluoride to an ammonium species[11]). Proton sponge,[12] being highly hindered, is not likely to form an ammonium intermediate, yet due to its pronounced basicity, is more effective than NMM. The latter, unhindered around the nitrogen atom, is more effective than the stronger base DIEA. Possible interference towards the formation of an ammonium species in the case of 2- or 6-substituted pyridine could rationalise the differences between (*a*) 2- and 4-picoline and (*b*) 2,6- and 3,4-lutidine. A discussion of the competition between basic and nucleophilic catalysis during acylation reactions is found in ref. 11*c*. Although there is currently no direct evidence that acid fluorides are converted to ammonium salts in the presence of tertiary amines, the fact that DMAP is a more efficient catalyst than stronger bases has been rationalised, in other cases, by the ease with which it yields such ammonium species.[9] With Fmoc-Val-F the model tests were extended to the acylation of a polymeric benzyl alcohol (Wang resin[13]) using a variety of solvent systems and bases (Table 2).‡

It is clear that the loading of hydroxy resins proceeds best in solvents of low polarity such as $CH_2Cl_2$, toluene or THF. Again, in spite of its high basicity and its perceived tendency toward inducing side reactions, DMAP was shown to be the most effective base for loading purposes. Other bases gave far lower resin substitutions. Relative to previously reported anchoring *via* acid fluorides in the presence of the polar cosolvent pyridine[7,8] the Wang resin was completely loaded within 10 min at much lower racemisation levels when DMAP was used in non-polar media. Presumably because the reactions are over quickly the DMAP technique is relatively safe from racemisation problems.

Based on these model studies the method was extended to other proteinogenic, side chain protected amino acids and to sterically hindered substrates such as MeVal (*N*-methylvaline) and Aib (amino isobutanoic acid) (Table 3). In most cases complete loading could be achieved with little, if any, racemisation being detected by the highly sensitive GC-MS method.[14] Problems arose in the case of Fmoc-His(Trt)-F which is somewhat unstable on storage and is poorly soluble in non polar solvents such as toluene and $CH_2Cl_2$. A convenient method of handling histidine *via* Fmoc-His(Trt)-NCA has recently been reported.[15] An amino acid which is very sensitive toward racemisation is cysteine and the use of Fmoc-Cys(But)-F led to 5.9% of the D-amino acid using DMAP. On the other hand the weaker, hindered base collidine[16] effected high loading with little loss of configuration (0.05%).

Previously[4] it was shown that the use of a strong organic base such as DMAP for resin loading led to premature Fmoc deblocking with subsequent dipeptide formation on the resin. This potential side reaction was checked for Fmoc-Cys(But)-OH under the normal conditions used (0.15 mol $dm^{-3}$ in toluene, 2 equiv. DMAP), and it was found that Fmoc-cleavage

**Table 1** Model acylations of benzyl alcohol by Boc-Ala-F in the presence of various bases

| Base | $pK_a$ ($H_2O$) | $t_{1/2}$/min |
|---|---|---|
| DMAP | 9.6 | < 2 |
| DBU | 12 | 5 |
| Proton sponge | 12.1 | 10 |
| DIEA | 10.1 | 18.5 |
| NMM | 7.4 | 15.8 |
| Collidine | 7.4 | 28 |
| 2,6-Lutidine | 6.6 | 90 |
| 3,4-Lutidine | 6.5 | 13 |
| 2-Picoline | 5.9 | 75 |
| 4-Picoline | 6.0 | 29 |
| Pyridine | 5.2 | 72 |
| Isoquinoline | 5.4 | 40 |
| Quinoline | 4.8 | 187.5 |

**Table 2** Anchoring of Fmoc-Val-OH *via* Fmoc-Val-F (3 equiv., coupling concentration: 0.15 mol $dm^{-3}$) to *p*-alkoxy benzyl alcohol-resin

| Base (equiv.) | Solvent | Reaction time/min | Final resin substitution/ mmol $g^{-1}$ | % D-Content[a] |
|---|---|---|---|---|
| 40% Pyridine | $CH_2Cl_2$ | 30 | 0.42 | < 0.01 |
| Pyridine (2) | Toluene | 30 | 0.19 | — |
| DIEA (2) | Toluene | 30 | 0.33 | — |
| NNM (2) | Toluene | 30 | 0.56 | — |
| DMAP (2) | Toluene | 10 | 0.75 | < 0.01 |
| DMAP (2) | Toluene | 2 × 30 | 0.76 | — |
| Pyridine (2) | DMF | 30 | 0.00 | — |
| DIEA (2) | DMF | 30 | 0.08 | — |
| NMI (2) | DMF | 30 | 0.09 | — |
| DMAP (2) | DMF | 30 | 0.66 | 2.66 |
| DMAP (2) | THF | 10 | 0.76 | 0.12 |
| DMAP (1) | $CH_2Cl_2$ | 10 | 0.46 | — |
| DMAP (2) | $CH_2Cl_2$ | 10 | 0.76 | < 0.01 |

[a] The detection limit was determined to be 0.01% with a signal to noise ratio of > 3 : 1.

**Table 3** Anchoring *via* Fmoc amino acid fluorides (3 equiv., coupling concentration: 0.15 mol $dm^{-3}$) to *p*-alkoxy benzyl alcohol resin

| Amino acid fluoride | Base (equiv.) | Solvent | Reaction time/min | Final resin substitution/ mmol/g | % D-Content |
|---|---|---|---|---|---|
| Fmoc-Gly-F | DMAP (2) | $CH_2Cl_2$ | 30 | 0.747 | — |
| Fmoc-Ala-F | DMAP (2) | Toluene | 30 | 0.757 | <0.01 |
| Fmoc-Ala-F | DMAP (2) | DMF | 30 | 0.757 | 0.11 |
| Fmoc-Asp(OBu$^t$)-F | DMAP (2) | $CH_2Cl_2$ | 30 | 0.750 | <0.01 |
| Fmoc-Glu(OBu$^t$)-F | DMAP (2) | $CH_2Cl_2$ | 30 | 0.741 | <0.01 |
| Fmoc-Ile-F | DMAP (2) | THF | 30 | 0.753 | 0.05 |
| Fmoc-Ile-F | DMAP (2) | $CH_2Cl_2$ | 2 × 90 | 0.760 | <0.01 |
| Fmoc-Leu-F | DMAP (2) | $CH_2Cl_2$ | 30 | 0.751 | <0.01 |
| Fmoc-Lys(BOC)-F | DMAP (2) | $CH_2Cl_2$ | 30 | 0.746 | <0.01 |
| Fmoc-Met-F | DMAP (2) | THF | 30 | 0.757 | 0.17 |
| Fmoc-Met-F | DMAP (2) | Toluene | 30 | 0.748 | 0.42 |
| Fmoc-Pro-F | DMAP (2) | $Ch_2Cl_2$ | 30 | 0.748 | <0.01 |
| Fmoc-Phe-F | DMAP (2) | Toluene | 30 | 0.750 | <0.01 |
| Fmoc-Ser(Bu$^t$)-F | DMAP (2) | Toluene | 30 | 0.754 | <0.01 |
| Fmoc-Thr(Bu$^t$)-F | DMAP (2) | Toluene | 30 | 0.737 | 0.09 |
| Fmoc-Trp-F | DMAP (2) | $CH_2Cl_2$ | 30 | 0.759 | <0.01 |
| Fmoc-Tyr(Bu$^t$)-F | DMAP (2) | $CH_2Cl_2$ | 30 | 0.754 | <0.01 |
| Fmoc-Aib-F | DMAP (2) | Toluene | 30 | 0.756 | — |
| Fmoc-NMeVal-F | DMAP (2) | $CH_2Cl_2$ | 30 | 0.758 | <0.01 |
| Fmoc-Cys(Bu$^t$)-F | DMAP (2) | Toluene | 30 | 0.759 | 5.9 |
| Fmoc-Cys(Bu$^t$)-F | Collidine (2) | Toluene | 30 | 0.681 | 0.05 |

was very slow (dibenzofulvene detected by HPLC < 0.2% after 1 h; 1.7% after 16 h). These results were confirmed by ES-MS examination of the crude material obtained after cleavage of the loaded amino acid from the resin. No dipeptide could be detected.

In conclusion, while DMAP might have been considered a risky base for resin loading on the basis of earlier studies, success in the present work is presumably due to the conditions chosen: short time treatment in a non-polar medium. The lack of side reactions and the high reactivity of the fluorides in non-polar solvents make them well suited for the effective loading of hydroxy-functionalised resins in peptide synthesis.

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## Footnotes

† In each case 0.2 ml $CDCl_3$ solutions of Boc-Ala-F (0.315 mol $dm^{-3}$), benzyl alcohol (0.315 mol $dm^{-3}$) and organic base (0.157 mol $dm^{-3}$) were mixed and the resulting reaction monitored by $^1H$ NMR. Reaction was considered half over when integration of the benzyl protons of the alcohol ($\delta$ = 4.58) equalled those of the ester ($\delta$ = 5.12). $[\alpha]_D^{23.5} = -27$ (*c* = 0.1, EtOAc).

‡ For determination of resin substitution the Fmoc-group was cleaved with 20% piperidine–DMF for 30 min and the piperidine–dibenzofulvene adduct determined by measuring the UV absorbance at 301 nm. The highest resin loading was 0.76 mmol $g^{-1}$ as determined by different coupling methods using double acylations (2 × 90 min) and a large excess of the acylating agent.

Racemisation was examined as follows: the Fmoc-deblocked amino acids were cleaved from the resin (50% TFA/DCM, 2 h), transformed into the corresponding *N*-trifluoracetyl amino acid isopropyl esters and analysed on a chiral GC column.[14]

## References

1 S. S. Wang, *J. Org. Chem.*, 1975, **40**, 1235.
2 J. K. Chang, M. Shimizu and S. S. Wang, *J. Org. Chem.*, 1976, **41**, 3255.
3 E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard and B. J. Williams, *J. Chem. Soc. Chem. Commun.*, 1978, 537.
4 E. Atherton, C. J. Logan and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. I*, 1981, 538.
5 E. Atherton, N. L. Benoiton, E. Brown, R. C. Sheppard and B. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1981, 336.
6 L. A. Carpino, D. Sadat-Aalaee, H.-G. Chao and R. H. DeSelms, *J. Am. Chem. Soc.*, 1990, **112**, 9651.
7 K. Akaji, N. Kuriyama, T. Kimura, Y. Fujwara, Y. Kiso, *Tetrahedron Lett.*, 1992, **33**, 3177.
8 J. Green and K. Bradley, *Tetrahedron*, 1993, **49**, 4141.
9 For a review regarding the use of 4-(dialkylamino) pyridines as acylation catalysts see: G. Höfle, W. Steglich and H. Vorbrüggen, *Angew. Chem.*, 1978, **90**, 602.
10 S. C. Mayer and M. M. Joullie, *Syn. Commun.*, 1994, **24**, 2367.
11 (*a*) A. I. Kirichenko, L. M. Litvinenko, I. N. Dotsenko, N. G. Kotenko, E. Nikkelsen and V. D. Berestetskaya, *Dokl. Acad. Nauk SSSR*, 1979, **244**, 1125; (*b*) A. K. Sheinkman, S. I. Suminov and A. N. Kost, *Russ. Chem. Rev.*, 1973, **42**, 642; (*c*) V. Gold and E. G. Jefferson, *J. Chem. Soc.*, 1953, 1409.
12 (*a*) R. W. Alder, P. S. Bowman, W. R. S. Steele, and D. R. Winterman, *J. Chem. Soc., Chem. Commun.*, 1968, 723; (*b*) H. A. Staab and T. Saupe, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 865.
13 S. S. Wang, *J. Am. Chem. Soc.*, 1973, **95**, 1328.
14 S. Kusumoto, M. Matsukura and T. Shiba, *Biopolymers*, 1981, **20**, 1869.
15 Y.-F. Zhu, R. K. Blair and W. D. Fuller, *Tetrahedron Lett.*, 1994, **35**, 4673.
16 L. A. Carpino and A. El-Faham, *J. Org. Chem.*, 1994, **59**, 695.