Peptide Assembly in the Absence of Base *via* Fmoc Amino Acid Fluorides

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Fmoc amino acid fluorides are highly efficient reagents for peptide assembly in the absence of tertiary bases, the presence of which may cause a variety of side reactions.

Recently, Fmoc amino acid fluorides have been shown to be efficient, rapid-acting coupling reagents for both solution- and solid-phase synthesis, being particularly useful in the case of hindered amino acids such as those bearing α,α-dialkyl substituents.[1,2] Relative to the analogous acid chlorides, protected amino acid fluorides are doubly unique: (*a*) shelf-stable derivatives can be obtained even in the case of amino acids bearing *tert*-butyl-based side-chain protecting groups and (*b*) oxazolone formation in the presence of tertiary organic bases is not observed.[1a]

In the present communication, a third unique difference between acid chlorides and acid fluorides is described, namely the ability of the latter to effect efficient coupling in the absence of base. Amine acylation *via* acid halides is traditionally carried out in the presence of one equivalent of base in the belief that neutralization of the released hydrohalic acid is essential. Previously, two-phase couplings of protected amino acid fluorides were effected in the presence of $NaHCO_3$ or Na_2CO_3[3] and one-phase solution or solid-phase syntheses *via* a tertiary organic amine such as *N,N*-diisopropylethylamine (DIEA).[2] Unfortunately, the presence of any base other than the amino acid or peptide ester is a possible source of additional racemization, and it is indeed generally noted that loss of chirality parallels the nature and extent of base present.[4] In addition, for the specific case of Fmoc protection there is a danger of premature deblocking,[5] especially in the case of slow coupling reactions.[2]

Relative to the amine salts of the other hydrohalic acids the hydrofluoride salts are quite unusual. For example simple aliphatic amines readily bind three equivalents of HF as illustrated by the case of $Et_3N{\cdot}3HF$, a stable commercially available reagent which is distillable without decomposition at atmospheric pressure.[6] It, therefore, might be expected that acid fluoride coupling could proceed efficiently in the presence of only a fraction of an equivalent of a tertiary amine. In fact, for the difficult coupling of Fmoc-Aib-F (Aib = amino isobutanoic acid) with H-Aib-OMe, little difference was observed for runs carried out in the presence of 0.5, 1 or 2 equiv. of DIEA (Fig. 1) although reaction proceeded somewhat faster and nearer to completion in the latter cases.† More surprisingly, the reaction proceeded at similar rates in the complete absence of any added tertiary amine. In contrast, comparison of analogous reactions of Fmoc-Val-F and Fmoc-Val-Cl showed that the chloride reacted up to a maximum of about 50%, indicating that half of the free amino functions had been neutralized and rendered unavailable by reaction with the hydrogen chloride released (Fig. 2).‡

As test sequences for application of no base/acid fluoride coupling alamethicin F30 and ACP(65-69) were chosen for assembly *via* solid phase and rapid Fmoc/TAEA (TAEA = Tris(2-aminoethyl)amine) solution syntheses, respectively. Alamethicin F30 is rich in sterically hindered Aib residues and previously only the acid fluoride technique proved successful for its assembly *via* the solid phase approach.[4] Although only single couplings (30 min) were performed, alamethicin was also obtained by the no base approach in excellent yield and purity as shown by HPLC analysis [Fig. 3(*a*)] and MS.§ The quality of the crude product was similar to that recently obtained in the presence of one equivalent of DIEA.[4] The rapid solution synthesis of pentapeptide **1**. (the N-terminal half of the ACP decapeptide[5b]) was carried out in the absence of base in a manner analogous to syntheses described earlier[1a,3] with each coupling step being executed by direct addition of the appropriate Fmoc-amino acid fluoride to the growing peptide in dichloromethane solution. Following completion of the coupling reaction (30 min), TAEA was added directly to the reaction mixture prior to buffer extraction. The deblocked pentapeptide was obtained in high quality and good yield as verified by HPLC [Fig. 3(*b*)] and MS analysis.¶

H-Val-Gln-Ala-Ala-Ile-OH
1

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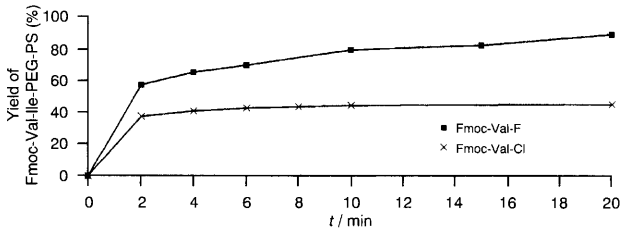

Fig. 2 Formation of Fmoc-Val-Ile-PEG-PS from Fmoc-Val-F or Fmoc-Val-Cl and Ile-PEG-PS

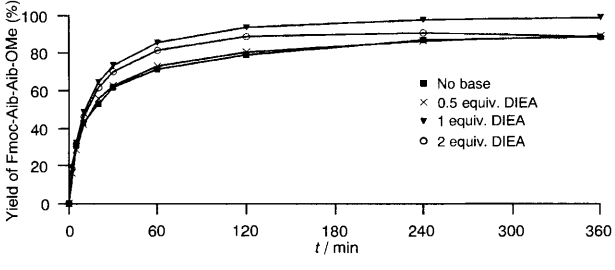

Fig. 1 Formation of Fmoc-Aib-Aib-OMe from Fmoc-Aib-F and H-Aib-OMe using different concentrations of base

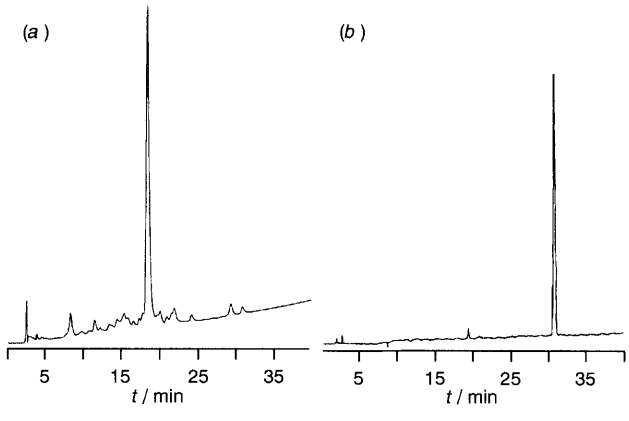

Fig. 3 Solid phase synthesis of alamethicin F30 (*a*) and rapid solution phase synthesis of ACP (65-69); (*b*) using the no base/acid fluoride approach

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Footnotes

† In mechanistic studies of the amination of simple acid fluorides Satchell and coworkers[7] have shown the reaction to be catalysed by excess amine or an added tertiary amine. These workers also drew attention to the fact that the aminolysis of acid fluorides has more in common with the analogous reaction of active esters than of acid chlorides, bromides or iodides.

‡ Hydrogen fluoride is significantly less acidic than hydrogen chloride in Me_2SO[8] and while measurements in DMF appear not to have been made the relative acidities in the two solvents are expected to be similar.[9]

§ Synthesis of alamethicin F30 (Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Pheol) was performed on a Milligen 9050 peptide synthesizer; resin: *o*-Cl-Trt-resin (Novabiochem) was loaded with Fmoc-Pheol (0.24 mmol g^{-1}), couplings: single couplings, 30 min, 4.5 equiv. of Fmoc amino acid fluorides, peptide-resin cleavage: 2% triisopropylsilane, 5% water, 5% phenol, 50% TFA–CH_2Cl_2, 45 min. ES-MS data of crude peptides synthesized: alamethicin F30: calcd. for $[M + H]^+$: 1964.3, found: 1964.3.

¶ Synthesis of ACP(65-69) was carried out without isolation of any intermediates. On a 2 mmol scale 20 ml of dichloromethane was used as solvent and 11 ml of TAEA for the deblocking step. Coupling was allowed to proceed for 30 min, deblocking for 15 min. The free pentapeptide was characterized by HPLC (Fig. 3) and ES-MS (calcd. for $[M + H]^+$: 501.6 found: 501.5), yield 74%.

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