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Fmoc Amino Acid Fluorides in Peptide Synthesis - Extension of the Method to Extremely Hindered Amino Acids

Holger Wenschuh,[*a,b] Michael Beyermann,[a] Rüdiger Winter,[a] Michael Bienert, [a]

Dumitru Ionescu [c] and Louis A. Carpino [c]

[a] Forschungsinstitut für Molekulare Pharmakologie, A.- Kowalke Str. 4., D-10315 Berlin, Germany, [b] Max-Planck-Institut für Infektionsbiologie, Monbijou Str. 2, D-10117 Berlin, Germany and [c] Department of Chemistry, University of Massachusetts, Amherst, MA 01003, USA.

Fmoc amino acid fluorides were found to be exceptionally well suited for the coupling of extremely hindered amino acids using a new strategy involving treatment with bis(trimethylsilyl)acetamide prior to the acylation step.

Fmoc amino acid fluorides have recently been shown to be excellent coupling reagents for both solution and solid phase peptide synthesis[1] and for efficient acylation of hydroxy functions[2]. However, their most impressive application is for the coupling of adjacent sterically hindered amino acids such as Aib[3] as demonstrated by the first successful solid phase synthesis of the peptaibols[4], naturally occurring peptides containing a high content (up to 60%) of Aib residues. In order to evaluate their scope and limitations, acid fluorides have now been applied to very bulky couplings. Initial studies involved coupling of the very hindered α,α-dialkylated and N-methylated amino acid fluorides (Iva, Deg, NMeGly, NMeVal, NMeAib) with the moderately hindered amino group of Aib-OMe (Fig. 1). (coupling conditions: HClxAibOMe 0.5 mmol, 0.55 mmol Fmoc amino acid fluoride, 1.05 mmol DIEA, 5 ml DMF). Fmoc amino acid fluorides not previously described were prepared using the method of Carpino[1a] and Kaduk[5], respectively.

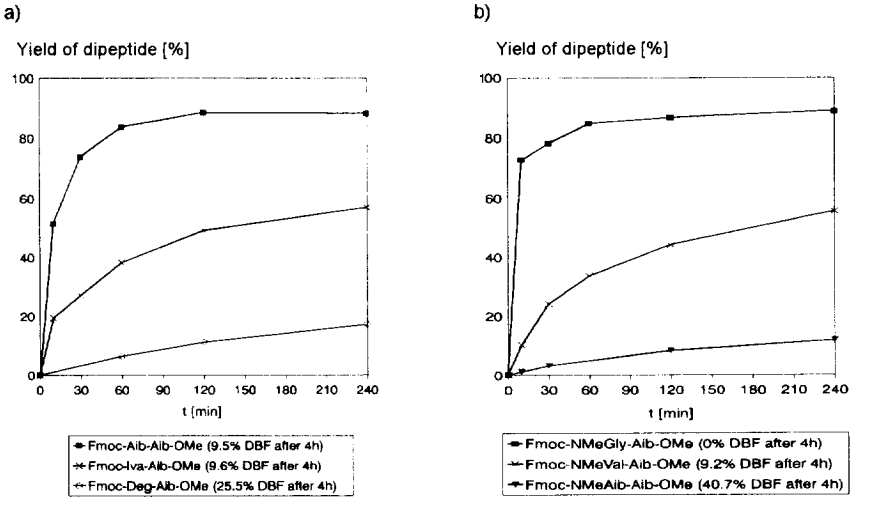

Figure 1.: Coupling of increasingly hindered Fmoc amino acid fluorides to Aib-OMe.

While the coupling of acid fluorides derived from Aib and NMe-Gly proceeded without difficulty, problems arose when more hindered residues were used. Thus, the speed of acylation was found to be significantly decreased when Fmoc-Iva-F and Fmoc-NMeVal-F were used. The even more hindered systems Deg and NMe-Aib gave only small amounts of the desired dipeptides (Fmoc-Deg-Aib-OMe: 18%; Fmoc-NMeAib-Aib-OMe: 12%). When the coupling rate was particularly low as in these two cases, premature deblocking of the Fmoc group was observed as a prominent side reaction. In addition, IR studies revealed that Fmoc-amino acid fluorides derived from α,α-dialkylated species are converted slowly into the corresponding oxazolones when tertiary amines are present. The most extensive conversion to oxazolone was found in the case of Deg (ca. 15% after 4h).

Since fluorides of proteinogenic amino acids suffer little conversion to oxazolone[1a] under the same conditions the increased reactivity in the case of more hindered systems can be ascribed to the known "gem dialkyl effect"[6]. Thus avoidance of conditions which promote such side reactions, e.g. the use of tertiary amines in polar solvent systems, should be advantageous for couplings involving very hindered residues. Very recently it was reported that Fmoc amino acid fluorides can be coupled even in the complete absence of any base[7] and therefore the coupling of Fmoc-Deg-F and Fmoc-NMeAib-F to Aib-OMe were repeated in DCM in the absence of DIEA (Fig. 2.). The HCl salt of the amino component was first converted into the corresponding HF salt. Although in fact Fmoc-deprotection was significantly reduced the coupling rate was very slow in both cases. Although the addition of 1 equivalent of DIEA accelerated the rate of coupling without causing additional Fmoc-deprotection the yield of dipeptide did not rise above 20%, after 2 h.

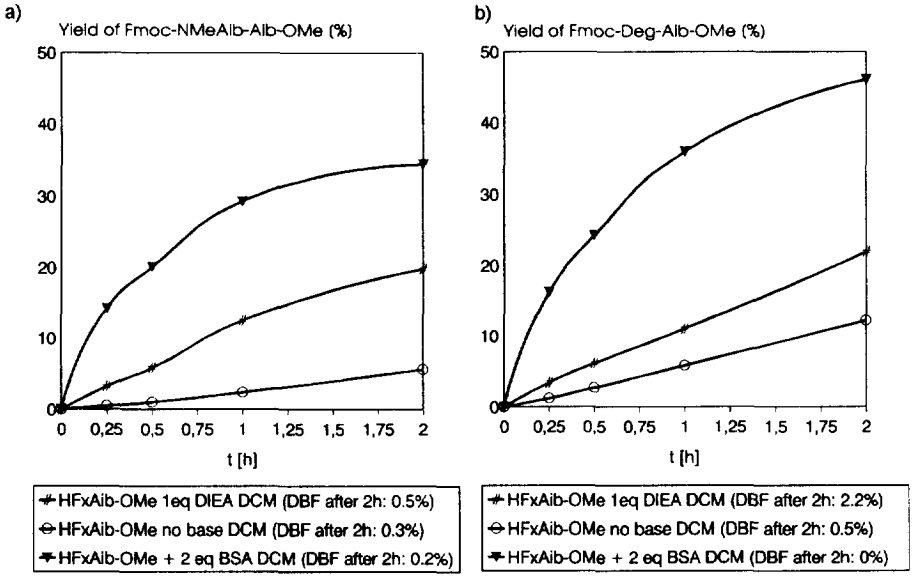

Figure 2.: Coupling of Fmoc-NMeAib-F (a) and Fmoc-Deg-F (b) to Aib-OMe under different conditions.

A more effective approach involved prior treatment of the amino component of the coupling system with a silylating agent such as BSA[8]. Recently, it was found that amide bonds can be formed readily under mild conditions by reaction of N-silylamines with acyl fluorides[9], even in the case of sterically hindered secondary amines[10].

In this communication an investigation of the reactivity of Fmoc-protected amino acid fluorides following silylation of very hindered amino acid derivatives is described. Although ^{1}H-NMR studies showed that silylation was complete after

5 min at room temperature[11], in these first studies silylation was allowed to proceed overnight prior to adding the acid fluoride. Under these conditions increased rates of formation of dipeptide were observed. Interestingly, the addition of BSA protected the Fmoc amino acid from premature Fmoc deblocking as indicated by the small amount of DBF detected by HPLC analysis.

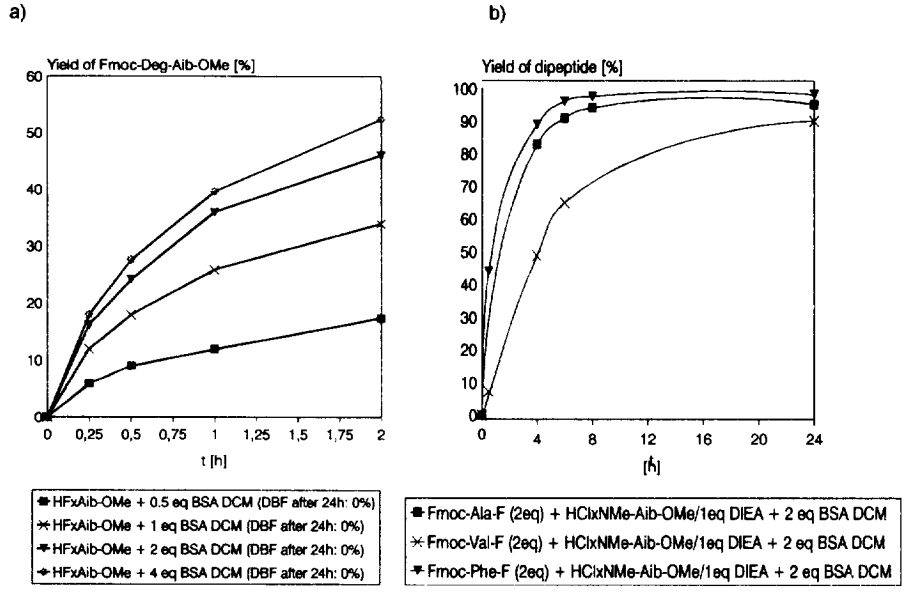

Figure 3.: a) Coupling of Fmoc-Deg-F to Aib-OMe using varying amounts of BSA b) Coupling of Fmoc amino acid fluorides to NMeAib-OMe.

As shown in Fig. 3a the amount of BSA added roughly parallels the speed of reaction. In order to confirm the results the method was extended to coupling onto the even more hindered amino group of NMeAib, which was earlier shown to be extremely difficult. Previously only high temperatures (50 °C) or long reaction times (7 days) in combination with large excess of an activated amino acid derivative (e.g. 5 eq. BOC-Phe-NCA) led to adequate reaction rates[12].
Again when silylation of the methyl ester (2 eq. BSA overnight in DCM) preceded coupling, high dipeptide yields were obtained (Fig.3b) after relatively short reaction times (conditions: 2 eq. Fmoc-amino acid fluorides, 0.3 M in DCM).
In conclusion, it is clear that under appropriate conditions Fmoc amino acid fluorides are very effective for the incorporation of even extremely hindered amino acids such as Iva, Deg or NMeAib into peptides.

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References: Abbreviations: BSA = N,O-bis(trimethylsilyl) acetamide, DBF = dibenzofulvene, DIEA = diisopropylethylamine, Aib = α-amino isobutyric acid, Deg = α,α-diethylglycine, Iva =isovaline = α- ethylalanine.

1. a.) L.A. Carpino, D. Sadat-Aalaee, H.G. Chao, R.H. DeSelms, *J. Am. Chem. Soc.* **1990**, *112*, 9651. b.) J.-N. Bertho, A. Loffet, C. Pinel, F. Reuther, G. Sennyey, *Tetrahedron Lett.* **1991**, *32*, 1303.
2. D. Granitza, M. Beyermann, H. Wenschuh, H. Haber, L.A. Carpino, G.A. Truran, M. Bienert, *J. Chem. Soc. Chem. Commun.* **1995**, 2223.
3. H. Wenschuh, M. Beyermann, E. Krause, M. Brudel, R. Winter, M. Schümann, L.A. Carpino, M. Bienert, *J. Org. Chem.* **1994**, *59*, 3275.
4. H. Wenschuh, M. Beyermann, H. Haber, J.K. Seydel, E. Krause, M. Bienert, L.A. Carpino, A. El-Faham, F. Albericio, *J. Org. Chem.* **1995**, *60*, 405.
5. C. Kaduk, H. Wenschuh, M. Beyermann, K. Forner, L.A. Carpino, M. Bienert *Letters in Peptide Science* **1996**, 2, 285. Analytical data for new Fmoc amino acid fluorides: Fmoc-NMeGly-F: Yield: 68% as a white solid, MP: 94-96 °C, IR: 1857.5 cm^{-1}, Anal. Calcd. for $C_{18}H_{16}NO_3F$: C, 69.00; H, 5.15; N, 4.47. Found: C, 69.29; H, 5.04; N, 4.58. Fmoc-NMeVal-F: Yield: 73% as a white solid, MP: 158-160 °C, IR: 1834.5 cm^{-1}, Anal. Calcd. for $C_{21}H_{22}NO_3F$: C, 70.97; H, 6.24; N, 3.94. Found: C, 70.74; H, 6.52; N, 3.98. Fmoc-NMeAib-F: Yield: 64% as a white solid, MP: 174-178 °C, IR: 1827.7 cm^{-1}, Anal. Calcd. for $C_{20}H_{20}NO_3F$: C, 70.36; H, 5.91; N, 4.10. Found: C, 70.81; H, 6.42; N, 3.91. Fmoc-Deg-F: Yield: 74% as a white solid, IR: 1834.9 cm^{-1}, Anal. Calcd. for $C_{21}H_{22}NO_3F$: F, 5.35. Found: F, 5.31. ES-MS: calcd.: 356.4 $[M+H]^+$, found: 356.2 $[M+H]^+$.
6. P.G. Sammes, D.J. Weller *Synthesis* **1995**, 1205.
7. H. Wenschuh, M. Beyermann, A. El-Faham, S. Ghassemi, L.A. Carpino, M. Bienert, *J. Chem. Soc. Chem. Commun.* **1995**, 669.
8. In a typical experiment 0.1 mmol of the amino acid methyl ester was dissolved in 0.2 ml of dry DCM. Molecular sieves (100 mg) were present for protection against hydrolysis. BSA was added through a septum and the resulting mixture stirred overnight. Fmoc amino acid fluoride was dissolved in 0.4 ml of dry DCM and added through the septum. HPLC and ES-MS were used for product analysis: ES-MS: Fmoc-Aib-Aib-OMe, calcd.: 447.5 $[M+Na]^+$, found: 447.3 $[M+Na]^+$, Fmoc-Iva-Aib-OMe, calcd.: 461.3 $[M+Na]^+$, found: 461.2 $[M+Na]^+$, Fmoc-Deg-Aib-OMe, calcd.: 475.1 $[M+Na]^+$, found: 475.4 $[M+Na]^+$, Fmoc-NMeGly-Aib-OMe, calcd.: 433.5 $[M+Na]^+$, found: 433.3 $[M+Na]^+$, Fmoc-NMeVal-Aib-OMe, calcd.: 475.5 $[M+Na]^+$, found: 475.3 $[M+Na]^+$, Fmoc-NMeAib-Aib-OMe, calcd.: 461.3 $[M+Na]^+$, found: 461.3 $[M+Na]^+$, Fmoc-Ala-NMeAib-OMe, calcd.: 447.5 $[M+Na]^+$, found: 447.5 $[M+Na]^+$, Fmoc-Phe-NMeAib-OMe, calcd.: 523.7 $[M+Na]^+$, found: 523.4 $[M+Na]^+$,Fmoc-Val-NMeAib-OMe,calcd.: 475.5 $[M+Na]^+$, found: 475.4 $[M+Na]^+$.
9. S. Rajeswari, R.J. Jones, M. P. Cava, *Tetrahedron Letters* **1987**, *28*, 5099. Because Fmoc amino acid fluorides of α,α- disubstituted amino acids suffer ready conversion to the corresponding oxazolones in the presence of fluoride ion acting as base the Cava methodology is not viable for the systems under study. On the other hand an in situ process involving BSA and believed to be initiated by silylation of the amino acid ester leads to increased reactivity. Mechanistic aspects of this reactivity enhancement are under investigation.
10. H.-O. Kim, B. Gardner, M. Kahn, *Tetrahedron Letters* **1995**, *36*, 6013.
11. NMR data for silylated amino functions: *$(CH_3)_3Si$-Aib-OMe:* ^{1}H-NMR (ppm, $CDCl_3$): 1.38 (s, 6, $C(C\underline{H}_3)_2$); 3.72 (s, 3, $OC\underline{H}_3$), *$(CH_3)_3Si$-NMeAib-OMe:* ^{1}H-NMR (ppm, CD_2Cl_2): 1.36 (s, 6, $C(C\underline{H}_3)_2$); 1.95 (s, 3, $NC\underline{H}_3$); 3.70 (s, 3, $OC\underline{H}_3$). reference line: TMS 0 ppm.
12. J.R. Spencer, V.V. Antonenko, N. G.J. Delaet, M. Goodman *Int. J. Pept. Prot. Res.* **1992**, *40*, 282.

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