

CHAPTER 2

Methods for Removing the Fmoc Group

Gregg B. Fields

1. Introduction

The electron withdrawing fluorene ring system of the 9-fluorenylmethyloxycarbonyl (Fmoc) group renders the lone hydrogen on the β -carbon very acidic and, therefore, susceptible to removal by weak bases (1,2). Following the abstraction of this acidic proton at the 9-position of the fluorene ring system, β -elimination proceeds to give a highly reactive dibenzofulvene intermediate (1–5). Dibenzofulvene can be trapped by excess amine cleavage agents to form stable adducts (1,2). The stability of the Fmoc group to a variety of bases (6–10) is reported in Table 1. The Fmoc group is, in general, rapidly removed by primary (i.e., cyclohexylamine, ethanolamine) and some secondary (i.e., piperidine, piperazine) amines, and slowly removed by tertiary (i.e., triethylamine [Et₃N], *N,N*-diisopropylethylamine [DIEA]) amines. Removal also occurs more rapidly in a relatively polar medium (*N,N*-dimethylformamide [DMF] or *N*-methylpyrrolidone [NMP]) compared to a relatively nonpolar one (dichloromethane [DCM]). During solid-phase peptide synthesis (SPPS), the Fmoc group is removed typically with piperidine, which in turn scavenges the liberated dibenzofulvene to form a fulvene-piperidine adduct. Standard conditions for removal include 30% piperidine-DMF for 10 min (11), 20% piperidine-DMF for 10 min (12,13), 55% piperidine-DMF for 20 min (14), 30% piperidine in toluene-DMF (1:1) for 11 min (11,15–17), 23% piperidine-NMP for 10 min (9), and 20% piperidine-NMP for 18 min (18). Piperidine-DCM should not be utilized, since an amine salt precipitates after relatively brief stand-

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Table 1
Removal of the Fmoc Group

Compound	Base	Solvent	Time, min	Deprotection, %	Reference
Fmoc-Gly-PS	10% Morpholine	DCM	240	18 ^a	6
Fmoc-Gly-PS	10% Morpholine	DMF	240	75 ^a	6
Fmoc-Gly-PS	50% Morpholine	DCM	240	100 ^a	6
Fmoc-Val	50% Morpholine	DMF	1	50 ^b	7
Fmoc-Ala-OtBu	50% Morpholine	DCM	120	100 ^c	8
Fmoc-Gly-PS	10% Piperidine	DCM	240	100 ^a	6
Fmoc-Val	20% Piperidine	DMF	0.1	50 ^b	7
Fmoc-Gly-HMP-PS	23% Piperidine	NMP	0.25	50 ^d	9
Fmoc-Ala-OtBu	50% Piperidine	DCM	<5	100 ^c	8
Fmoc-Val	5% Piperazine	DMF	0.33	50 ^b	7
Fmoc-Ala-OtBu	50% Piperazine	DCM	60	100 ^c	8
Fmoc-PCA	59% 1,4-bis-(3-aminopropyl)piperazine	CDCl ₃	2	100 ^e	10
Fmoc-Val	50% Dicyclohexylamine	DMF	35	50 ^b	7
Fmoc-Ala-OtBu	50% Dicyclohexylamine	DCM	>1080	100 ^c	8
Fmoc-Val	50% DIEA	DMF	606	50 ^b	7
Fmoc-Ala-OtBu	50% DIEA	DCM	>1080	100 ^c	8
Fmoc-Val	10% 4-Dimethylaminopyridine	DMF	85	50 ^b	7

Fmoc-Ala-O <i>t</i> Bu	50% DBU	DCM	<5	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	50% Pyrrolidine	DCM	<5	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	50% Cyclohexylamine	DCM	<5	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	50% Ethanolamine	DCM	<5	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	50% Diethylamine	DCM	180	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	50% Triethylamine	DCM	>1080	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	50% Ammonia	DCM	>1080	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	50% Tributylamine	DCM	>1080	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	1.0 mM triethylenediamine	DCM	>1080	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	1.0 mM Hydroxylamine HCl	DCM	>1080	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	0.5 mmol Proton sponge	DCM	>1080	100 ^c	8
Fmoc-Ala-O <i>t</i> Bu	2.0 mmol NaOH	30% CH ₃ OH- <i>p</i> -dioxane	<5	100 ^c	8
Fmoc-PCA	50% Tris(2-aminoethyl)amine	CDCl ₃	2	100 ^e	10
Fmoc-PCA	59% 1,3-Cyclohexanebis-(methylamine)	CDCl ₃	2	100 ^e	10

^aDeprotection of Fmoc-Gly-PS was quantitated spectrophotometrically at 273 nm (6)

^bDeprotection of Fmoc-Val was quantitated by amino acid analysis (7)

^cDeprotection of Fmoc-Ala-O-*t*Bu was quantitated by thin-layer chromatography (8)

^dDeprotection of Fmoc-Gly-HMP-PS was quantitated by ninhydrin analysis (9).

^eDeprotection of 9-fluorenylmethyl *N*-*p*-chlorophenyl carbamate (Fmoc-PCA) was quantitated by ¹H-NMR (10). Dibenzofulvene was scavenged in 2 min by tris(2-aminoethyl)amine, 15 min by 1,3-cyclohexanebis-(methylamine), and 50 min by 1,4-bis-(3-aminopropyl)piperazine.

ing (11). An inexpensive alternative to piperidine for Fmoc removal is diethylamine, with standard conditions being 60% diethylamine-DMF for 180 min (19,20) or 10% diethylamine-*N,N*-dimethylacetamide (DMA) for 120 min (21,22).

2. Monitoring

Fmoc removal can be monitored spectrophotometrically because of the formation of dibenzofulvene or fulvene-piperidine adducts. Monitoring is especially valuable in "difficult" sequences, where Fmoc removal may be slow or incomplete (17,23,24). Slow deprotection has been correlated to a broad fulvene-piperidine peak detected at 312 nm (24–26). Monitoring of a broad fulvene-piperidine peak at 365 nm has been used to demonstrate slow deprotection from Fmoc-(Ala)₅-Val-4-hydroxymethylphenoxy (HMP)-copoly(styrene-1%-divinylbenzene)-resin (PS); in turn, detection of a narrow fulvene-piperidine peak demonstrated efficient deprotection of the same sequence on a different solid support (HMP-polyethylene glycol-PS) (27). Monitoring of fulvene-piperidine at 313 nm was utilized during the successful synthesis of the entire 76-residue sequence of ubiquitin (28). Dibenzofulvene formation has been monitored at 270 or 304 nm (29).

3. Side Reactions

Repetitive piperidine treatments can result in a number of deleterious side reactions, such as diketopiperazine and aspartimide formation and racemization of esterified Cys derivatives. Base-catalyzed cyclization of resin-bound dipeptides to diketopiperazines is especially prominent in sequences containing Pro, Gly, D-amino acids, or *N*-methyl amino acids. For continuous-flow Fmoc SPPS, diketopiperazine formation is suppressed by deprotecting for 1.5 min with 20% piperidine-DMF at an increased flow rate (15 mL/min), washing for 3 min with DMF at the same flow rate, and coupling the third Fmoc-amino acid *in situ* with benzotriazolyl *N*-oxytrisdimethylaminophosphonium hexafluorophosphate (BOP), 4-methylmorpholine, and 1-hydroxybenzotriazole (HOBt) in DMF (30). For batch-wise SPPS, rapid (a maximum of 5 min) treatments by 50% piperidine-DMF should be used, followed by DMF washes and then *in situ* acylations mediated by BOP or 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (31). Piperidine catalysis of aspartimide formation from side-

chain-protected Asp residues can be rapid, and is dependent on the side-chain-protecting group. Treatment of Asp(OBzl)-Gly, Asp(OcHex)-Gly, and Asp(O*t*Bu)-Gly with 20% piperidine-DMF for 4 h resulted in 100, 67.5, and 11% aspartimide formation, respectively (32), whereas treatment of Asp(OBzl)-Phe with 55% piperidine-DMF for 1 h resulted in 16% aspartimide formation (33). The racemization of C-terminal-esterified Cys derivatives by 20% piperidine-DMF is also problematic, with D-Cys formed to the extent of 11.8% from Cys(Trt), 9.4% from Cys(Acm), 5.9% from Cys(*t*Bu), and 36.0% from Cys(S*t*Bu) after 4 h of treatment (34).

Some piperidine-catalyzed side-reactions may be minimized by using other bases to remove the Fmoc group. Two percent 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)-DMF, at a flow rate of 3 mL/min for 10 min, is used to minimize monodealkylation of either Tyr(PO₃Me₂) or Tyr(PO₃Bzl₂) (29). For example, 50% monodealkylation of Tyr(PO₃Me₂) occurred in 7 min with 20% piperidine-DMF, but required 5 h with 1M DBU in DMF, whereas 50% monodealkylation of Tyr(PO₃Bzl₂) occurred in 12 min with 20% piperidine-DMF and 14 h with 1M DBU in DMF (29). Racemization of esterified Cys(Trt) was reduced from 11.8% with 20% piperidine-DMF to only 2.6% with 1% DBU-DMF after 4 h of treatment (29,34). Unfortunately, aspartimide formation of Asp(O*t*Bu)-Asn is worse with DBU compared to piperidine (35). This reagent is recommended for continuous-flow syntheses only, since the dibenzofulvene intermediate does not form an adduct with DBU and thus must be washed rapidly from the peptide resin to avoid reattachment of dibenzofulvene (29). However, a solution of DBU-piperidine-DMF (1:1:48) is effective for batch syntheses, since the piperidine component scavenges the dibenzofulvene.

4. Glycopeptide Synthesis

The mild conditions of Fmoc chemistry are, in general, more suited for glycopeptide syntheses than Boc chemistry, because repetitive acid treatments can be detrimental to sugar linkages (36). However, some researchers prefer morpholine to piperidine as an Fmoc removal agent during glycopeptide SPPS, because the pK_a of morpholine (8.3) is lower than that of piperidine (11.1), and is thus less detrimental to side-chain glycosyls (36,37). Side-chain Ser and Thr glycosyls are stable to base deprotection by neat morpholine (38,39) for 30 min (40) and 50%

morpholine-DMF for 20–30 min (41–43). A 4-h treatment of Cys(Trt) with 50% morpholine-DMF resulted in 3.8% D-Cys, which is considerably less racemization than that seen with piperidine (34).

5. Solution Syntheses

For rapid solution-phase synthesis, it is desirable to use an Fmoc removal agent that forms a dibenzofulvene adduct that can be extracted in phosphate buffer (pH 5.5). Such an adduct is obtained when either 4-(aminomethyl)piperidine (44) or tris(2-aminoethyl)amine is used for Fmoc removal (10). Precipitates or emulsions can form during 4-(aminomethyl)piperidine-fulvene adduct extraction from a DCM layer, so tris(2-aminoethyl)amine is preferred (10). Complete deprotection and scavenging of 9-fluorenylmethyl *N*-*p*-chlorophenyl carbamate (Fmoc-PCA) (0.14 mmol) was achieved in 2 min with 2 mL of tris(2-aminoethyl)amine (100 Eq) in 2 mL CDCl_3 (10). Polymeric-bound amines, such as piperazine-PS (2.4 mEq/g) (45) and a copolymer of styrene, 2,4,5-trichlorophenyl acrylate, and *N,N'*-dimethyl-*N,N'*-bisacryloylhexamethylene diamine, with subsequent replacement of activated ester groups by 1-(2-aminoethyl)piperazine (3.3 mEq/g) (46), also efficiently remove the Fmoc group in solution-phase syntheses. The use of polymeric-bound amines allows for the isolation of the free amino component by simple filtration of the resin, since the polymer traps the dibenzofulvene (45,46).

6. Notes

1. Amine impurities that could possibly remove the Fmoc group include dimethylamine found in DMF (47) and methylamine found in NMP (48). Fmoc-Gly was found to be deprotected after 7 d in DMA, DMF, and NMP to the extent of 1, 5, and 14%, respectively (49). Although these rates of decomposition are considered extremely low, it is recommended that these solvents be freshly purified before use (26,47). The presence of HOBt (0.001–0.1M) greatly reduces the detrimental effect of methylamine (48,50) whereby Fmoc-Gly-HMP-PS was <1% deprotected after 20 h in NMP (48).
2. The primary and secondary amine lability of the Fmoc group also prompted an investigation of Fmoc removal by esterified or resin-bound amino acids. Fmoc-Ala and Fmoc-Gly (in DMF) were labile to Pro-*Or*Bu, where $t_{1/2} \sim 9$ and 7 h, respectively (51). Fmoc liberation was less rapid by Pro-Lys(4- NO_2 -Z)-Gly-OET ($t_{1/2} \sim 40$ and 35 h for Fmoc-Ala and Fmoc-Gly, respectively, in the presence of 1 Eq DIEA), and greatly reduced by the presence of HOBt (1 Eq) and 2,4-dinitrophenol (2 Eq) (51). The Fmoc group was

less labile to primary amino acid esters, even in the presence of DIEA (51). Fmoc-Leu (in DCM) was deprotected very slowly by Gly-PS, with $t_{1/2} = 300$ and 1500 h in the presence of 1.8 and 1.2 Eq of DIEA, respectively (8). These rates of Fmoc removal by Gly-PS are insignificant in SPPS.

3. There are several alternatives to base removal of the Fmoc group, such as fluoride ion or hydrogenation. Fmoc-Phe was rapidly deprotected (~ 2 min) by 0.05–0.1M tetrabutylammonium fluoride trihydrate (TBAF) in DMF (52). Continuous-flow Fmoc SPPS of Leu-Ala-Gly-Val, carried out with 20-min deprotections of 0.02M TBAF in DMF, resulted in a highly homogeneous crude product (52). Adding 100 Eq of MeOH to TBAF-DMF solutions could inhibit readdition of dibenzofulvene to the peptide resin and diketopiperazine formation (52). Succinimide formation from Asn, glutarimide formation from Gln, and the instability of benzyl ester groups are potential problems of TBAF deprotection (53,54). Complete deprotection of Fmoc-Ala (in CH₃OH), Fmoc-Gly (in 95% ethanol), and Fmoc-Leu (in 75% aqueous ethanol) by hydrogenation with 10% Pd-on-charcoal catalyst in the presence of acetic acid (two drops) occurred in 4, 22, and 4 h, respectively (55). Deprotection was solvent-dependent, with generation of Gly from Fmoc-Gly occurring with $t_{1/2} \sim 30$ h in 20% acetic acid-CH₃OH, $t_{1/2} \sim 17$ h in DMF, and $t_{1/2} \sim 7$ h in DMF containing 2 Eq of DIEA by hydrogenation with 10% Pd-on-charcoal catalyst (49). Fairly rapid Fmoc-Gly deprotection in DMF ($t_{1/2} \sim 2.5$ h) was found when Pd(OAc)₂ was used as the catalyst instead of Pd-on-charcoal (49). Studies with Fmoc-Gly-OBzl showed selective removal of the benzyl ester in the presence of the Fmoc group by hydrogenation in CH₃OH with 10% Pd-BaSO₄ catalyst for ~ 1 h (56).

References

1. Carpino, L. A. and Han, G. Y. (1972) The 9-fluorenylmethoxycarbonyl amino-protecting group. *J. Org. Chem.* **37**, 3404–3409.
2. Carpino, L. A. (1987) The 9-fluorenylmethoxycarbonyl family of base-sensitive amino-protecting groups. *Acc. Chem. Res.* **20**, 401–407.
3. O'Ferrall, R. A. M. and Slae, S. (1970) β -elimination of 9-fluorenylmethanol in aqueous solution: an *E1cB* mechanism. *J. Chem. Soc. (B)*, 260–268.
4. O'Ferrall, R. A. M. (1970) β -elimination of 9-fluorenylmethanol in solutions of methanol and *t*-butyl alcohol. *J. Chem. Soc. (B)*, 268–274.
5. O'Ferrall, R. A. M. (1970) Relationships between *E2* and *E1cB* mechanisms of β -elimination. *J. Chem. Soc. (B)*, 274–277.
6. Merrifield, R. B. and Bach, A. E. (1978) 9-(2-Sulfo)fluorenylmethoxycarbonyl chloride, a new reagent for the purification of synthetic peptides. *J. Org. Chem.* **43**, 4808–4816.
7. Atherton, E., Logan, C. J., and Sheppard, R. C. (1981) Peptide synthesis, part 2: procedures for solid-phase synthesis using *N* $^{\alpha}$ -fluorenylmethoxycarbonylamino-

- acids on polyamide supports: synthesis of substance P and of acyl carrier protein 65-74 decapeptide. *J. Chem. Soc. Perkin Trans. I*, 538-546.
8. Chang, C.-D., Waki, M., Ahmad, M., Meienhofer, J., Lundell, E. O., and Haug, J. D. (1980) Preparation and properties of N^{α} -9-fluorenylmethoxycarbonyl-amino acids bearing *tert*.-butyl side chain protection. *Int. J. Peptide Protein Res.* **15**, 59-66.
 9. Harrison, J. L., Petrie, G. M., Noble, R. L., Beilan, H. S., McCurdy, S. N., and Culwell, A. R. (1989) Fmoc chemistry: synthesis, kinetics, cleavage, and deprotection of arginine-containing peptides, in *Techniques in Protein Chemistry* (Hugli, T. E., ed.), Academic, San Diego, pp 506-516
 10. Carpino, L. A., Sadat-Aalae, D., and Beyermann, M. (1990) Tris(2-aminoethyl)amine as a substitute for 4-(aminomethyl)piperidine in the FMOC/polyamine approach to rapid peptide synthesis. *J. Org. Chem.* **55**, 1673-1675.
 11. Albericio, F., Kneib-Cordonier, N., Biancalana, S., Gera, L., Masada, R. I., Hudson, D., and Barany, G. (1990) Preparation and application of the 5-(4-(9-fluorenylmethoxycarbonyl)aminomethyl-3,5-dimethoxyphenoxy)valeric acid (PAL) handle for the solid-phase synthesis of C-terminal peptide amides under mild conditions. *J. Org. Chem.* **55**, 3730-3743.
 12. Atherton, E., Fox, H., Harkiss, D., Logan, C. J., Sheppard, R. C., and Williams, B. J. (1978) A mild procedure for solid phase peptide synthesis: use of fluorenylmethoxycarbonylamino-acids. *J. Chem. Soc., Chem Commun* 537-539.
 13. Atherton, E., Fox, H., Harkiss, D., and Sheppard, R. C. (1978) Application of polyamide resins to polypeptide synthesis: an improved synthesis of β -endorphin using fluorenylmethoxycarbonylamino-acids. *J. Chem. Soc., Chem Commun* 539,540
 14. Chang, C.-D., Felix, A. M., Jimenez, M. H., and Meienhofer, J. (1980) Solid-phase peptide synthesis of somatostatin using mild base cleavage of N^{α} -fluorenylmethoxycarbonylamino acids. *Int. J. Peptide Protein Res.* **15**, 485-494.
 15. Hudson, D. (1988) Methodological implications of simultaneous solid-phase peptide synthesis 1: comparison of different coupling procedures. *J. Org. Chem.* **53**, 617-624
 16. Otvos, L., Jr., Urge, L., Hollosi, M., Wroblewski, K., Graczyk, G., Fasman, G. D., and Thurin, J. (1990) Automated solid-phase synthesis of glycopeptides: incorporation of unprotected mono- and disaccharide units of *N*-glycoprotein antennae into T cell epitopic peptides. *Tetrahedron Lett.* **31**, 5889-5892.
 17. Fontenot, J. D., Ball, J. M., Miller, M. A., David, C. M., and Montelaro, R. C. (1991) A survey of potential problems and quality control in peptide synthesis by the fluorenylmethoxycarbonyl procedure. *Peptide Res.* **4**, 19-25.
 18. Fields, G. B. and Fields, C. G. (1991) Solvation effects in solid-phase peptide synthesis. *J. Am. Chem. Soc.* **113**, 4202-4207.
 19. Sivanandaiah, K. M., Gurusiddappa, S., and Babu, V. V. S. (1988) Peptides related to leucine-/methionine-enkephalinamides: synthesis and biological activities. *Indian J. Chem.* **27B**, 645-648.
 20. Sivanandaiah, K. M., Gurusiddappa, S., Channe Gowda, D., and Suresh Babu, V. V. (1989) Improved solid phase synthesis of luteinizing hormone releasing hormone analogues using 9-fluorenylmethoxycarbonyl amino acid active esters and

- catalytic transfer hydrogenation with minimal side-chain protection and their biological activities. *J. Biosci.* **14**, 311–317.
21. Butwell, F. G. W., Haws, E. J., and Epton, R. (1988) Advances in ultra-high load polymer supported peptide synthesis with phenolic supports 1: a selectively-labile C-terminal spacer group for use with a base-mediated N-terminal deprotection strategy and Fmoc amino acids. *Makromol. Chem., Macromol. Symp.* **19**, 69–77.
 22. Butwell, F. G. W., Epton, R., McLaren, J. V., Small, P. W., and Wellings, D. A. (1985) Gel phase ^{13}C n.m.r. spectroscopy as a method of analytical control in ultra-high load solid (gel) phase peptide synthesis with special reference to LH-RH, in *Peptides: Structure and Function* (Deber, C. M., Hruby, V. J., and Kopple, K. D., eds.), Pierce Chemical Co., Rockford, IL, pp. 273–276.
 23. Larsen, B. D., Larsen, C., and Holm, A. (1991) Incomplete Fmoc-deprotection in solid phase synthesis, in *Peptides 1990* (Giralt, E. and Andreu, D., eds.), Escom, Leiden, The Netherlands, pp. 183–185.
 24. Atherton, E. and Sheppard, R. C. (1985) Detection of problem sequences in solid phase synthesis, in *Peptides: Structure and Function* (Deber, C. M., Hruby, V. J., and Kopple, K. D., eds.), Pierce Chemical Co., Rockford, IL, pp. 415–418.
 25. Sheppard, R. C. (1988) True automation of peptide synthesis. *Chem. Britain* **24**, 557–562.
 26. Atherton, E. and Sheppard, R. C. (1989) *Solid Phase Peptide Synthesis: A Practical Approach*. IRL, Oxford, UK.
 27. Barany, G., Solé, N. A., Van Abel, R. J., Albericio, F., and Selsted, M. E. (1992) Recent advances in solid-phase peptide synthesis, in *Innovation and Perspectives in Solid Phase Synthesis—1992* (Epton R., ed.), Intercept, Andover, UK, pp. 29–38.
 28. Ogunjobi, O. and Ramage, R. (1990) Ubiquitin: Preparative chemical synthesis, purification and characterization. *Biochem. Soc. Trans.* **18**, 1322–1333.
 29. Wade, J. D., Bedford, J., Sheppard, R. C., and Tregear, G. W. (1991) DBU as an N^α -deprotecting reagent for the fluorenylmethoxycarbonyl group in continuous flow solid-phase peptide synthesis. *Peptide Res.* **4**, 194–199.
 30. MilliGen/Biosearch (1990) Flow rate programming in continuous flow peptide synthesizer solves problematic synthesis. *MilliGen/Biosearch Report* **7**, 4–5, 11. MilliGen/Biosearch Division of Millipore, Bedford, MA.
 31. Pedroso, E., Grandas, A., de las Heras, X., Eritja, R., and Giralt, E. (1986) Diketopiperazine formation in solid phase peptide synthesis using *p*-alkoxybenzyl ester resins and Fmoc-amino acids. *Tetrahedron Lett.* **27**, 743–746.
 32. Nicolás, E., Pedroso, E., and Giralt, E. (1989) Formation of aspartimide peptides in Asp-Gly sequences. *Tetrahedron Lett.* **30**, 497–500.
 33. Schön, I., Colombo, R., and Cseh, A. (1983) Effect of piperidine on benzylaspartyl peptides in solution and in the solid phase. *J. Chem. Soc., Chem. Commun.* 505–507.
 34. Atherton, E., Hardy, P. M., Harris, D. E., and Matthews, B. H. (1991) Racemization of C-terminal cysteine during peptide assembly, in *Peptides 1990* (Giralt, E. and Andreu, D., eds.), Escom, Leiden, The Netherlands, pp. 243, 244.
 35. Kitas, E. A., Knorr, R., Trzeciak, A., and Bannwarth, W. (1991) Alternative strategies for the Fmoc solid-phase synthesis of O^4 -phospho-L-tyrosine-containing peptides. *Helv. Chim. Acta* **74**, 1314–1328.

36. Kunz, H. (1987) Synthesis of glycopeptides: partial structures of biological recognition components. *Angew. Chem. Int. Ed. Engl.* **26**, 294–308.
37. Paulsen, H., Adermann, K., Merz, G., Schultz, M., and Weichert, U. (1988) Synthese von *O*-glycopeptiden. *Starch/Stärke* **40**, 465–472
38. Hoogerhout, P., Guis, C. P., Erkelens, C., Bloemhoff, W., Kerling, K. E. T., and van Boom, J. H. (1985) Synthesis of the glycotriptide alanyl-(*O*- β -D-galactopyranosyl-seryl)-alanine using (9-fluorenylmethoxy)carbonyl and esters of 2-(hydroxymethyl)-9,10-anthraquinone as temporary protecting groups. *Recl. Trav. Chim. Pays-Bas* **104**, 54–59.
39. Paulsen, H., Merz, G., and Weichert, U. (1988) Solid-phase synthesis of *O*-glycopeptide sequences. *Angew. Chem. Int. Ed. Engl.* **27**, 1365–1367.
40. Paulsen, H., Merz, G., Peters, S., and Weichert, U. (1990) Festphasensynthese von *O*-glycopeptiden. *Liebigs Ann. Chem.*, 1165–1173
41. Jansson, A. M., Meldal, M., and Block, K. (1990) The active ester *N*-Fmoc-3-*O*-[Ac₄- α -D-Manp-(1 \rightarrow 2)-Ac₃- α -D-Manp-1-]-threonine-*O*-Pfp as a building block in solid-phase synthesis of an *O*-linked dimannosyl glycopeptide. *Tetrahedron Lett* **31**, 6991–6994.
42. Peters, S., Bielfeldt, T., Meldal, M., Block, K., and Paulsen, H. (1991) Multiple column solid phase glycopeptide synthesis. *Tetrahedron Lett.* **32**, 5067–5070
43. Meldal, M. and Jensen, K. J. (1991) Pentafluorophenyl esters for the temporary protection of the α -carboxy group in solid phase glycopeptide synthesis. *J. Chem. Soc., Chem. Commun.* 483–485.
44. Beyermann, M., Bienert, M., Niedrich, H., Carpino, L. A., and Sadat-Aalae, D. (1990) Rapid continuous peptide synthesis via Fmoc amino acid chloride coupling and 4-(aminomethyl)piperidine deblocking. *J. Org. Chem.* **55**, 721–728
45. Carpino, L. A. and Williams, J. R. (1978) Polymeric de-blocking agents for the fluoren-9-ylmethoxycarbonyl (Fmoc) amino-protecting group. *J. Chem. Soc., Chem. Commun.* 450,451.
46. Arshady, R., Atherton, E., and Sheppard, R. C. (1979) Basic polymers for the cleavage of fluorenylmethoxycarbonyl amino-protecting groups in peptide synthesis. *Tetrahedron Lett.*, 1521–1524.
47. Stewart, J. M. and Young, J. D. (1984) *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chemical Co., Rockford, IL
48. Otteson, K. M., Harrison, J. L., Ligut, A., and Ashcroft, P. (1989) Solid phase peptide synthesis with *N*-methylpyrrolidone as the solvent for both Fmoc and Boc synthesis, in *Poster Presentations at the Eleventh American Peptide Symposium*, Applied Biosystems, Inc., Foster City, CA, pp. 34–38.
49. Atherton, E., Bury, C., Sheppard, R. C., and Williams, B. J. (1979) Stability of fluorenylmethoxycarbonylamino groups in peptide synthesis: cleavage by hydrogenolysis and by dipolar aprotic solvents. *Tetrahedron Lett.*, 3041,3042
50. Albercio, F. and Barany, G. (1987) Mild, orthogonal solid-phase peptide synthesis: use of *N* α -dithiasuccinoyl (Dts) amino acids and *N*-(iso-propylidithio) carbonylproline, together with *p*-alkoxybenzyl ester anchoring linkages. *Int. J. Peptide Protein Res.* **30**, 177–205.

- 51 Bodanszky, M., Deshmane, S. S., and Martinez, J. (1979) Side reactions in peptide synthesis. 11. Possible removal of the 9-fluorenylmethyloxycarbonyl group by the amino components during coupling. *J. Org. Chem.* **44**, 1622–1625.
52. Ueki, M. and Amemiya, M. (1987) Removal of 9-fluorenylmethyloxycarbonyl (Fmoc) group with tetrabutylammonium fluoride. *Tetrahedron Lett.* **28**, 6617–6620
- 53 Sieber, P (1977) Der 2-trimethylsilyläthyl-rest als selektiv abspaltbare carboxy-schutzgruppe. *Helv Chim Acta* **60**, 2711–2716
- 54 Mullen, D. G. and Barany, G. (1988) A new fluoridolizable anchoring linkage for orthogonal solid-phase peptide synthesis: design, preparation, and application of the *N*-(3 or 4)-[[4-(hydroxymethyl)phenoxy]-*tert*-butylphenylsilyl]phenyl pentanedioic acid monoamide (Pbs) handle. *J. Org. Chem.* **53**, 5240–5248.
55. Martinez, J , Tolle, J C , and Bodanszky, M. (1979) Side reactions in peptide synthesis 12 Hydrogenolysis of the 9-fluorenylmethyloxycarbonyl group. *J Org. Chem.* **44**, 3596–3598.
56. Rabanal, F., Haro, I., Reig, F., and Garcia-Anton, J. M. (1990) Estudio de la estabilidad del 9-fluorenilmetoxicarbonil frente a la hidrogenacion catalitica. *Anal. Quim* **86**, 84–88.