Peptide Synthesis via Amino Acid Halides†

LOUIS A. CARPINO,*,‡ MICHAEL BEYERMANN,*,§ HOLGER WENSCHUH,§ AND MICHAEL BIENERT§

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, and Forschungsinstitut für Molekulare Pharmakologie, A-Kowalke-Strasse 4, D-10315 Berlin, Germany

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Introduction

Progress in the field of peptide synthesis by both solution and solid phase techniques has always followed advances in the methodology of protecting the α-amino group and forming the peptide bond.[1] Both strands of research came together with the development of the 9-fluorenylmethyloxycarbonyl (Fmoc) group[2] which opened new avenues for the study of coupling methods. To one unfamiliar with the intricacies of peptide bond formation, the most obvious method of activating a carboxyl group for amide formation at room temperature or below would appear to be via a simple acid chloride.[3] In fact acid chlorides are rarely used and among peptide practitioners long ago gained the reputation of being "overactivated" and therefore prone to numerous side reactions including loss of configuration.[4] More recently, as highly efficient coupling procedures have been sought in order to handle hindered amino acids or aggregated[5] sequences, the search for appropriate coupling reagents has continued. Studies in our laboratories with these goals in mind are described in this Account. In spite of their supposed deficiencies, this work started with protected amino acid chlorides and then progressed to the more practical amino acid fluorides.

Historical Details

Although the use of protected amino acid chlorides in peptide bond formation goes back to the very first days of peptide synthesis, the practical utility of such reagents has been restricted to special situations for a number of reasons including the unpleasant nature of the reagents needed for their synthesis and their relatively poor shelf life. Fischer, in 1903, described the synthesis of *N*-carbethoxyglycine chloride and its reaction with amino acid esters to give dipeptides.[6] Unfortunately the carbethoxy group could not be removed without destroying the newly formed peptide bond so that further chain extension was impossible.

Real applications of acid chlorides to peptide synthesis had to await the discovery of the first amide bond-compatible amino-protecting group, the benzyloxycarbonyl ("carbobenzoxy", Z) function by Bergmann and Zervas[7] who applied the technique in a number of syntheses. For example, Z-Val-OH was converted to Z-Val-Cl (**1**) which could be purified by recrystallization and underwent reaction with amino acid esters within a few minutes without apparent stereomutation. It soon became clear that Z-amino acid chlorides showed only limited shelf stability, not primarily because of facile hydrolysis, although this can be a factor under poor storage conditions, but because of a more serious side reaction: spontaneous decomposition to the corresponding Leuchs anhydride (amino acid *N*-carboxyanhydride, NCA) **3** with loss of benzyl chloride (eq 1).[8] The more recently examined *tert*-butyloxycarbonyl (BOC) analogs are even less stable. The few recorded examples of the use of BOC-amino

Louis A. Carpino is Professor of Chemistry at the University of Massachusetts. He is the author of two previous Accounts (**1973**, *6*, 191 and **1987**, *20*, 401), the first of which contains a brief biographical note.

Michael Beyermann was born in 1953 in Berlin. He studied at the Humboldt University, obtaining his Ph.D. in 1979 on enzymatic protein semisynthesis. He then joined the Institute of Drug Research (now the Forschungsinstitut für Molekulare Pharmakologie, FMP). For the last 10 years he has worked on chemical methods of peptide synthesis and more recently has become interested in structure–activity studies of biologically active peptides. He is Head of the Peptide Synthesis Unit at the institute.

Holger Wenschuh was born in 1964 in Halle, Germany, obtaining his diploma in chemistry at the Friedrich Schiller University in Jena. In 1990 he joined the group of Dr. Michael Bienert at the FMP, obtaining his Ph.D. in 1995 on a project dealing with the use of protected amino acid fluorides in peptide synthesis. He worked at both the University of Massachusetts with Professor Carpino and at the Millipore Corp. with Dr. Fernando Albericio (University of Barcelona) on the same project. He is currently in the Peptide Synthesis Group at the Max-Planck-Institut für Infektionsbiologie, Berlin.

Michael Bienert was born in 1943 in the outskirts of Berlin. He obtained his Ph.D. at the Humboldt University in the field of alkaloid chemistry in 1969. He became a Research Associate at the Institute of Drug Research in 1971 and is currently Head of the Department of Peptide Pharmacology and Chemistry at the successor institute. His research interests include structure–activity studies of biologically active peptides and the development of improved methods of peptide synthesis and cyclization.

† Abbreviations used: ACP = acyl carrier peptide; Aib = α-aminoisobutyric acid; BOC = *tert*-butyloxycarbonyl; BSA = *N,O*-bis(trimethylsilyl)acetamide; *t*-Bu = *tert*-butyl; DAST = (diethylamino)sulfur trifluoride; DBF = dibenzofulvene; DCC = dicyclohexylcarbodiimide; DCM = dichloromethane; DIEA = *N,N*-diisopropylethylamine; DMAP = 4-(*N,N*-dimethylamino)pyridine; DMSO = dimethyl sulfoxide; DMF = *N,N*-dimethylformamide; Iva = α-ethylalanine; HOBt = *N*-hydroxybenzotriazole; HOPfp = pentafluorophenol; NCA = amino acid *N*-carboxyanhydride; PEG-PS = polyethylene glycol, polystyrene resin support for solid phase synthesis; py = pyridine; PyBroP = bromotripyrrolidinophosphonium hexafluorophosphate; TAEA = tris(2-aminoethyl)amine; TFFH = tetramethylfluoroformamidinium hexafluorophosphate; Ts = tosyl = *p*-toluenesulfonyl; UNCA = urethane-protected amino acid *N*-carboxyanhydride; Z = benzyloxycarbonyl.

‡ University of Massachusetts.

§ Forschungsinstitut für Molekulare Pharmakologie.

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(2) Carpino, L. A.; Han, G. Y. *J. Org. Chem*. **1972**, *37*, 3404.

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(4) Bodanszky, M. *Principles of Peptides Synthesis,* 2nd ed.; Springer-Verlag: Berlin, 1993; p 11.

(5) In solid phase peptide synthesis some couplings are particularly difficult in spite of the fact that the very same amino acids can be coupled readily in other sequences. The effect has been ascribed to sequence-dependent aggregation of the peptide chains. For recent discussions and references to earlier work see: (a) Mutter, M.; Altmann, K.-H.; Bellof, D.; Flörsheimer, A.; Herbert, J.; Huber, M.; Klein, B.; Strauch, L.; Vorher, T.; Gremlich, H.-U. Peptides. Structure and Function. In *Proceedings of the 9th American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; p 397. (b) Larsen, B. D.; Christensen, D. H.; Holm, A.; Zillmer, R.; Nielsen, O. F. *J. Am. Chem. Soc*. **1993**, *115*, 6247. (c) Narita, M.; Lee, J.−S.; Murakawa, Y.; Kojima, Y. *Bull. Chem. Soc. Jpn*. **1993**, *66*, 483.

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(8) Bergmann, M.; Zervas, L.; Schleich, H.; Leinert, F. *Z. Physiol. Chem*. **1932**, *212*, 72. Recently a convenient *in situ* method for the generation and use of Z-amino acid chlorides has been reported: Schmidt, U.; Kroner, M.; Beutler, U. *Synthesis* **1988**, 475.

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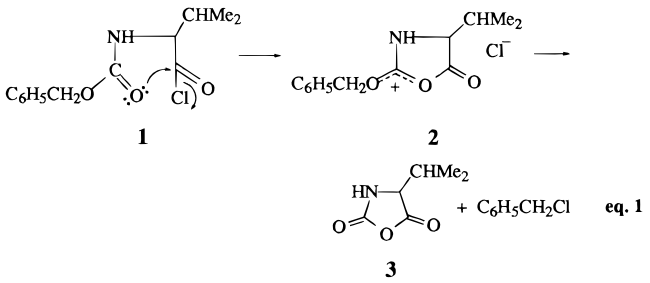

1 **2** **3**

+ $C_6H_5CH_2Cl$ **eq. 1**

acid chlorides have involved generation and use at very low temperatures (−30 to −20 °C),[9] or systems involving non-α amino acids.[10]

Recognizing the high potential reactivity of protected amino acid chlorides, Leplawy, Jones, Kenner, and Sheppard[11] sought to use such compounds in solving the difficult problem of coupling highly hindered amino acids such as α-aminoisobutyric acid (Aib). However, attempts to convert Z-Aib-OH (**4**) to the acid chloride gave only the corresponding oxazolone **6**. Such cyclizations are greatly facilitated by

4 **5** **6** **eq. 2**

the presence of the two α-methyl substituents ("*gem*-dimethyl effect").[12] The corresponding *N*-*p*-toluenesulfonyl (tosyl) derivative $TsNHCMe_2COCl$, unable to take part in such a reaction, could be readily obtained and was shown to be an effective acylating agent for Aib esters. This proved to be no general solution to the problem however since deblocking of the *N*-tosyl function[13] cannot be achieved under mild reaction conditions.[14]

In the case of simple *N*-acyl- as opposed to *N*-carbalkoxyamino acids, the problem of oxazolone formation is more serious since 2-alkyl- or 2-aryloxazolones are subject to facile stereomutation in contrast to the 2-alkoxy or 2-(aryloxy) analogs.[15] Evidence currently available on the structure of acid chlorides (or bromides) derived from such *N*-acylamino acids is not definitive, and it has been suggested that there may be an equilibrium between the acid halide and the isomeric oxazolone hydrohalide.[16] Products from reaction of *N*-(trifluoroacetyl)amino acids with thionyl chloride undergo reaction without loss of configuration and have been represented as normal acid chlorides.[17] The *N*-phthaloylamino acid halides cannot give oxazolones, and such compounds can also be used in peptide coupling without loss of configuration.[18]

Fmoc-Amino Acid Chlorides

For many years the only commonly used *N*-α protecting groups for peptide synthesis were the Z and BOC derivatives, and because of their very nature it was not possible to consider seriously the practical utilization of the corresponding acid chlorides. As noted in the Introduction, the situation changed following development of the Fmoc group as α-protectant.[2,19] Being highly stable under acidic conditions and not readily subject to S_N1 or S_N2 displacement reactions at the 9-fluorenylmethyl residue, the Fmoc amino acids appeared to be ideal substrates for conversion to the corresponding acid chlorides and their subsequent utilization in peptide synthesis. Following the first evidence[20] for the *in situ* generation and use of such intermediates, all of the common amino acids lacking polar side chains[21] as well as a number of those bearing benzyl-[21] or allyl-based[22] side chain protection were converted to stable, crystalline acid chlorides, some of which are now commercially available.[23] As expected, amino acids bearing *tert*-butyl-protected side chains could not generally be accommodated. In some cases (Asp, Glu) the appropriate esters could not be obtained due to facile conversion to cyclic anhydrides with loss of the *tert*-butyl residue.[24] In other cases (Tyr, Ser, Thr) while appropriate derivatives could be obtained, their shelf stability appeared insufficient for practical utilization.[25] Whether the slow degradation of these derivatives on standing is due to the presence of impurities left over from their synthesis or to some inherent instability is not yet clear.

By following the very rapid coupling via Fmoc-amino acid chlorides with an equally rapid deblocking step involving the polybasic amine 4-(aminomethyl)piperidine (4-AMP)[26] or preferably tris(2-aminoethyl)amine (TAEA),[27] it was possible to devise a rapid, stepwise solution synthesis of short peptides (Scheme 1, X = Cl) without the isolation of any intermediates. Deblocking is over in a few minutes, and the dibenzofulvene (DBF) adduct (**8** for the TAEA case) can be selectively extracted by means of a buffer of pH 5.5, leaving in the organic phase only the growing peptide ready for the next coupling step. This simple technique has now been used in the synthesis of numerous short peptides. An advantage of carrying out such

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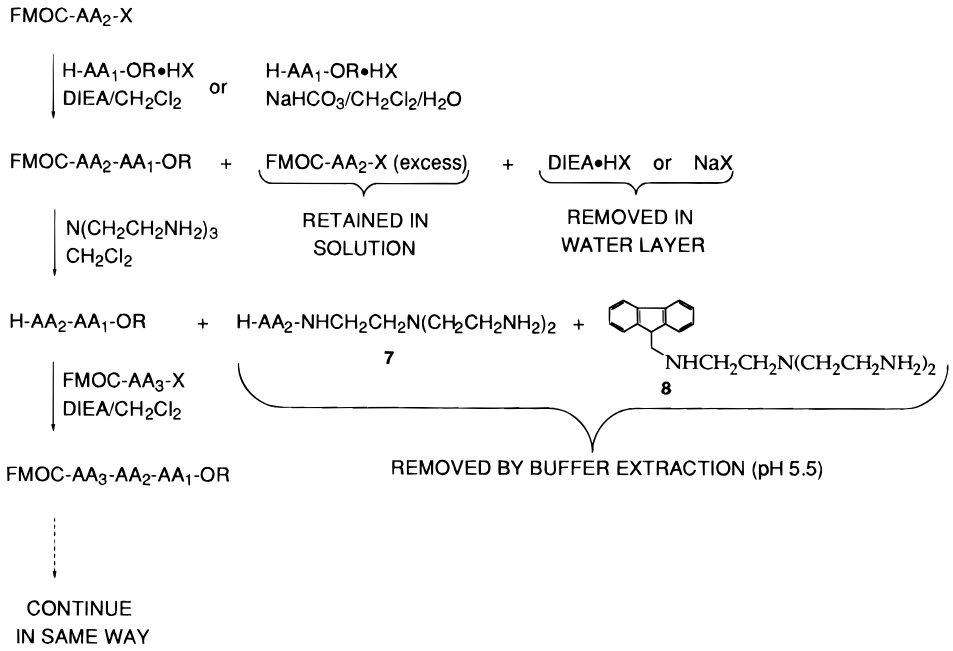
Scheme 1

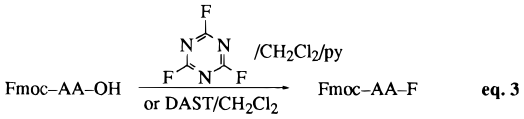


reactions under two-phase conditions is that reaction of the amino nucleophile at the acid chloride carbonyl function competes effectively with oxazolone formation (Scheme 1). For reactions carried out in homogeneous solution and under solid phase conditions, the unfortunate property of acid chlorides of being converted to oxazolones by the tertiary amines which are essential components of such systems has limited attempts to use these species as efficient coupling reagents. For best results under these conditions a hindered base such as 2,6-di-*tert*-butylpyridine can be used as hydrogen chloride acceptor since conversion to oxazolone is slow with such bases.[25] An alternate technique, demonstrated to be applicable also to solid phase syntheses, involves the use of ammonium[25] or potassium[28] salts of *N*-hydroxybenzotriazole (HOBt) as bases.

An impressive example involved the solid phase assembly of a group of hindered secondary amino acids.[29] For a particularly difficult coupling where the acid chloride itself led to a yield of only 50%, the efficiency could be increased if the reaction is carried out in the presence of AgCN. The exact mechanism of AgCN catalysis was not determined although it was shown that AgCN effected conversion of the chloride to the corresponding oxazolone, which in the presence of HCN, formed in the same process, underwent reaction more rapidly than the acid chloride itself.[30]

Fmoc-Amino Acid Fluorides

Because of the efficiency and orthogonality of the Fmoc/*t*-Bu strategy, a serious deficiency of the acid chloride technique is its inability to handle *t*-Bu-protected side chains. In contrast, the corresponding acid *fluorides*[24,31] suffer no such limitation. The acid fluorides are generally available via reaction of the acid with cyanuric fluoride in the presence of pyridine according to the general technique of Olah (eq 3).



eq. 3

More recently (diethylamino)sulfur trifluoride (DAST) has been shown to be a convenient reagent for this conversion in the absence of base.[32] The Fmoc-amino acid fluorides are equally stable in the case of amino acids bearing *t*-Bu, BOC, or *N*-trityl[33] side chain protection. Other advantages of the acid fluorides relative to the chlorides include their greater stability toward water, including moisture in the air, and their relative lack of conversion to the corresponding oxazolones on treatment with tertiary organic bases which had previously limited attempts to use the acid chlorides directly in solid phase peptide synthesis. While there is infrared evidence which is consistent with the formation of traces of oxazolone on treatment of Fmoc-protected proteinogenic amino acid fluorides, *e.g.*, Fmoc-Val-F with DIEA, this reaction is not rapid enough to compete with the direct acylation process. On the other hand, for systems prone to undergo cyclization readily, such as the α,α-diphenylglycine derivative, conversion to oxazolone occurs within minutes. Mechanistically, although the reactions of acid fluorides and acid chlorides show some superficial similarities, upon closer examination they are seen to be quite different. The older literature contains contrasting reports of the greater reactivity of one or

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the other species.[34] The acid fluorides are clearly less reactive toward neutral oxygen nucleophiles such as water or alcohols.[35,36] Recently, following extensive mechanistic studies, Satchell[37] suggested that the reactions of acid fluorides show more resemblance to those of active esters than of acid halides (Cl, Br, I). This view is consonant with the relative pK_a values in water of the various model precursor acids: HBr (−9), HCl (−8), HF (3.2), pentafluorophenol (HOPfp) (5.5).[38]

The properties of Fmoc-amino acid fluorides have made them ideal intermediates for both solution (Scheme 1, X = F) and solid phase peptide syntheses. In contrast to all other common coupling systems, these derivatives are useful even in the case of hindered amino acids. Excellent solid phase syntheses were demonstrated for ACP(65−74) (a decapeptide sequence of the acyl carrier protein), magainin-II-amide, and h-CRH (human corticotropin−releasing hormone), three models which are considered to be difficult sequences.[45] However, these systems are not so difficult as to preclude solid phase assembly, and in fact efficient syntheses have been described previously using any number of appropriate coupling reagents.[46] A more demanding test would involve application to a very highly hindered system such as one which incorporates two or more consecutive α-aminoisobutyric acid (Aib) units. Since adjacent Aib units induce helix formation,[47] the incorporation of such residues in naturally occurring peptides is potentially useful in exploring their special structure-inducing effects on biological activity. In pursuing studies of the effect of conformational changes within an assumed loop region of CRH, an analog having four consecutive Aib residues in positions 32−35 was targeted. Initial model studies involved comparison of the fluoride technique with methods previously reported to be exceptionally well suited for the incorporation of sterically hindered amino acids, such as the symmetric anhydride,[48] urethane-protected amino acid *N*-carboxyanhydride (UNCA),[49] and PyBroP[50] methods. Attempts to elongate the C-terminal hexapeptide of h-CRH by four Aib units showed that only the fluoride technique provides the desired decapeptide (Aib32−35)-h-CRH(32−41).[45] Synthesis of the whole sequence (Aib32−35)-h-CRH(1−41) also proceeds smoothly and results in a peptide of excellent quality.

Synthesis of the Naturally Occurring Peptaibols

Success in the synthesis of these Aib-containing CRH analogs suggested that the same general method might also be applied to a very interesting class of naturally occurring peptides, hitherto relatively unavailable, the so-called peptaibols. These unusual peptides are characterized by a high content of α,α-dialkylamino acids, such as Aib or α-ethylalanine (Iva). In the natural materials the N-terminus is generally acetylated, and the C-terminus is occupied by an amino alcohol, such as phenylalaninol (Pheol) or valinol (Valol). In view of their membrane-modifying properties the peptaibols have excited intense interest, yet in spite of being short peptides, consisting of approximately 20 amino acid units, they have only been synthesized by tedious procedures which combine carefully chosen stepwise solution techniques with chemical or enzymatic segment condensations.[51,52] The solid phase approach has been totally unsuccessful.[52]

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(35) Anion formation from an alcohol provides for potent acceleration. See: Mayer, S. C.; Joullié, M. M. *Synth. Commun.* **1994**, *24*, 2367.

(36) An interesting example was encountered during the study of 9-*cis*-retinoyl fluoride as an active site directed, specific mechanism-based inhibitor of opsin. Carrying the small fluorine atom in place of hydrogen, the acid fluoride strongly mimics the normal substrate 9-retinal, fitting neatly into the active site but reacting differently to achieve complete inactivation via amide bond formation. In this process hydroxyl and sulfhydryl nucleophiles as well as all amino groups other than that at the active site are unreactive. See: Wong, C. G.; Rando, R. *Biochemistry* **1984**, *23*, 20; *J. Am. Chem. Soc.* **1982**, *104*, 7374.

(37) Jedrzejczak, M.; Motie, R. E.; Satchell, D. P. N.; Satchell, R. S.; Wassef, W. N. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1471.

(38) These pK_a figures are misleading in that they refer to *dilute* aqueous solutions of HF. Pure HF, commonly used as a deblocking agent in Boc/benzyl solid phase peptide synthesis, is an extremely potent acid (H_o = ca. −11), and the same is true for *concentrated* aqueous solutions (40% HF, H_o = −2.05; 70% HF, H_o = −6.74). It is believed that while HF is highly dissociated in water, the resulting hydronium ion is deactivated by strong hydrogen bonding to fluoride ion as the tight ion pair $F^-\cdots H^+-OH_2$. As the HF concentration is increased, the F···HO hydrogen bond is replaced by the stronger FHF bond with consequent increasing dissociation ($H_3O^+F^- + HF \rightleftharpoons H_3O^+ + HF_2^-$).[39] In HF itself extensive aggregation occurs, again due to hydrogen bonding in the neutral species. At 25 °C, the average aggregate contains over three molecules of HF and polymers are present even in the gas phase. The reactivity of acid fluorides thus probably represents a balance between (a) the effect of the strength of the C−F bond which is high relative to that of C−X (Cl, Br, I),[40] leading to the usual designation of fluoride as a "poor" leaving group, and (b) the enhanced stabilization relative to the other halides of the intermediate formed by attack of a nucleophile at the carbonyl group by the strong C−F dipole induced by the high electronegativity of the fluoride atom.[41] A common example of the latter effect for a related nucleophilic substitution at unsaturated carbon is the pronounced reactivity of the Sanger reagent, 2,4-dinitrofluorobenzene, toward the amino function.[42] In DMSO, HF is very weakly acidic (pK_a = 15 ± 2),[43] and while the pK_a appears not to have been measured in DMF, the solvent used in most of the work described here, values in DMSO correlate well with those in the structurally similar solvent *N*-methylpyrrolidone.[44]

(39) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; Wiley-Interscience: New York, 1988; p 103.

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Chart 1

alamethicin F30:

Ac–Aib–Pro–Aib–Ala–Aib–Ala–Gln–Aib–Val–Aib–Gly–Leu–Aib–Pro–Val–**Aib–Aib**–Glu–Gln–Pheol

alamethicin F50:

Ac–Aib–Pro–Aib–Ala–Aib–Ala–Gln–Aib–Val–Aib–Gly–Leu–Aib–Pro–Val–**Aib–Aib**–Gln–Gln–Pheol

saturnisporin SA III:

Ac–Aib–Ala–Aib–Ala–**Aib–Aib**–Gln–Aib–Leu–Aib–Gly–**Aib–Aib**–Pro–Val–**Aib–Aib**–Gln–Gln–Pheol

trichotoxin A–50–J:

Ac–Aib–Ala–Aib–Leu–Aib–Gln–**Aib–Aib–Aib**–Ala–**Aib–Aib**–Pro–Leu–**Aib–Iva**–Gln–Valol

Difficulties arise because of steric effects which can be ascribed to the α,α-dialkylamino acids, such as stereomutation at the activated carboxylic acid residue,[53] incomplete coupling, or degradation inherent in the presence of acid-labile Aib−Pro linkages.[54]

Combination of the Fmoc-amino acid fluoride technique with a new application of an *o*-chlorotrityl-resin[55] for direct anchoring of Fmoc-protected amino alcohols makes possible stepwise solid phase assembly with subsequent cleavage from the resin under very mild conditions. Alamethicin F30 and F50, saturnisporin SA III, and trichotoxin A-50-J (Chart 1, with difficult coupling positions emphasized in boldface letters) were obtained by automated synthesis using a single-coupling protocol with 3−8 equiv each of Fmoc-amino acid fluoride and *N*,*N*-diisopropylethylamine.[56] Thirty-minute coupling times were used for all amino acids except Iva which was coupled for 2 h. According to RP-HPLC results, amino acid analysis, and mass spectrometric analysis, the crude peptides obtained were of remarkable quality. Examination of all four peptaibols for the presence of D-amino acids using the definitive method of Kusumoto et al.[57] showed that loss of stereochemical integrity could not have exceeded 0.8% for any of the chiral amino acids. These new applications of solid phase techniques open up the possibility of studying the unique properties of the peptaibols by ready access to a large number of substituted analogs.

In order to gain some feeling for the limitations of the acid fluoride method, systems more hindered than Aib were examined briefly. In spite of the fact that Aib−Aib coupling is slow for many activated amino acids, it can, as noted here, be easily achieved by acid fluoride reagents. While coupling of the highly hindered amino acid Iva to Aib resulted in significant dipeptide formation, the even more hindered NMeAib gave only small amounts of dipeptide after a coupling time of 4 h (Figure 1). The most serious problem which arises if sluggish couplings are attempted while using Fmoc protection involves premature deblocking of the activated amino acid derivative. Thus, for Fmoc-NMeAib-F, dibenzofulvene was released to the extent of 40.7% within 4 h.[58]

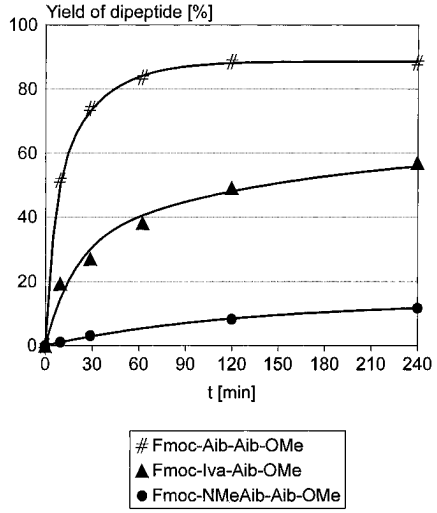

Figure 1. Coupling efficiency in the acylation of methyl α-aminoisobutyrate via Fmoc-Aib-F, Fmoc-Iva-F, and Fmoc-NMeAib-F, 0.2 M DMF.

No-Base Coupling of Fmoc-Amino Acid Fluorides

In early studies on the use of Fmoc-amino acid fluorides in peptide coupling and in the syntheses described above, it was assumed that at least 1 equiv of base would be required in order to bind the liberated HF and thereby achieve full acylation. In fact this proved not to be the case. Whereas reaction of an Fmoc-amino acid chloride with a free amino acid ester proceeded only to the extent of 50% as expected (Figure 2) since half of the amino acid ester is used up by formation of the unreactive hydrochloride salt, the corresponding reaction of the Fmoc-amino acid fluoride went to completion in the presence of 2, 1, or 0.5 equiv of DIEA. More remarkably, reaction proceeded only slightly less rapidly in the total absence

(53) Because of steric effects, coupling reactions which might normally be expected to occur quickly and cleanly have led to loss of stereochemical integrity upon coupling to hindered Aib units. For a general discussion of the relationship between loss of configuration during attack on an optically active oxazolone and the steric bulk of the reactive nucleophile (H-Gly-OEt < H-Ala-OMe < H-Aib-OMe), see: Goodman, M.; McGahren, W. J. *Tetrahedron* **1967**, *23*, 2031. For a specific example from the alamethicin series, see: Najaraj, R.; Balaram, P. *Tetrahedron* **1981**, *37*, 2001.

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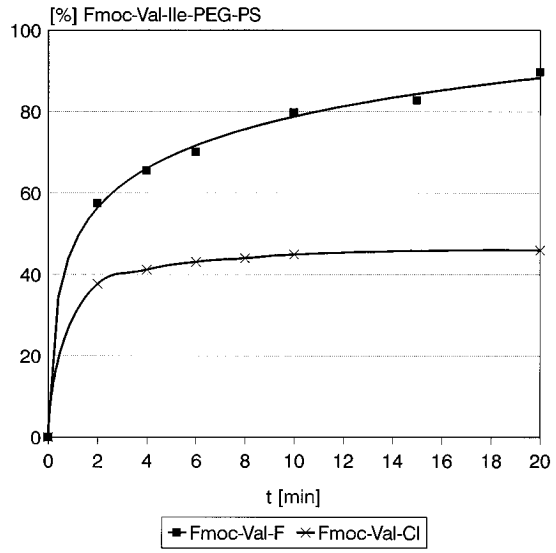

Figure 2. Time course for the formation of Fmoc-Val-Ile-PEG-PS from Fmoc-Val-F or Fmoc-Val-Cl and H-Ile-PEG-PS.

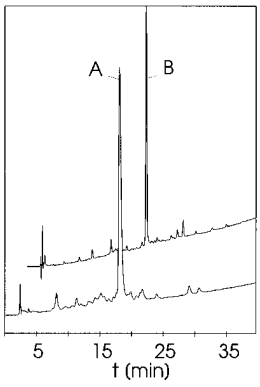

Figure 3. HPLC curves for the crude products obtained via solid phase syntheses of alamethicin F30 in the presence of (A) no base and (B) 1 equiv of DIEA. Single couplings for 30 min, 4.5 equiv of excess amino acid fluoride. Curve B displaced to the right.

of any added tertiary amine. The reaction rate was somewhat faster in DMF than in CH_2Cl_2.[59]

On the basis of these model studies, both stepwise solution and solid phase syntheses of various peptides have been carried out with acid fluorides in the absence of base. For example, rapid Fmoc/TAEA solution syntheses of the C-terminal ACP pentapeptide amide H-Asp-Tyr-Ile-Asn-Gly-NH_2 were carried out with and without base. The crude pentapeptide obtained in the absence of base was of significantly higher quality. Only 1.2 equiv of the acid fluoride was used in each coupling step.

Solid phase syntheses carried out in the absence of base included the assembly of alamethicin F30 and the partial sequence of h-CRH, (Aib32−35)-h-CRH-(32−41). Although only single couplings (30 min) were performed, both peptides were obtained by the no-base approach in excellent yield and purity (Figure 3). In addition, the no-base approach was successfully applied to the multiple syntheses of alamethicin analogs.[60] Because of their high solubility and stability for periods of up to at least 24 h in solvents such as

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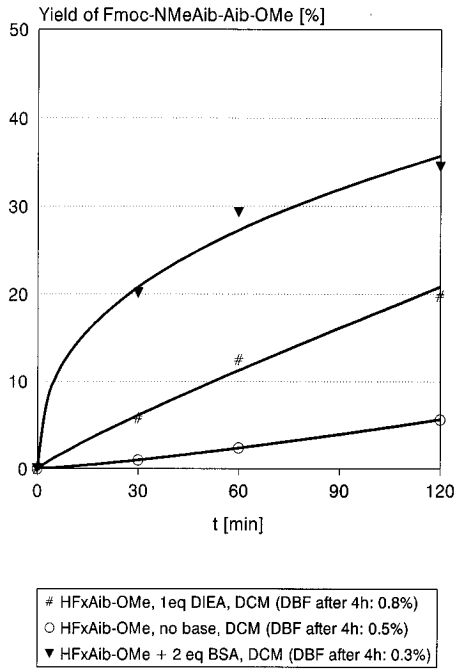


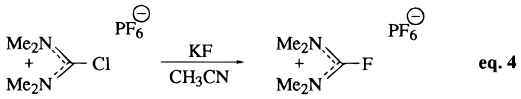
Figure 4. Time course for the reaction of Fmoc-NMeAib-F with the HF salt of H-Aib-OMe in the presence or absence of a silylating agent.

DMF, the Fmoc-amino acid fluorides clearly satisfy the requirements of automated instrumentation for multiple peptide synthesis which call for long-term storage of activated amino acids in an appropriate solvent.

In order to determine whether no-base coupling might be advantageous for the coupling of extremely hindered systems, coupling between Fmoc-NMeAib-F (1.2 equiv) and the hydrofluoride salt of H-Aib-OMe was examined. Premature Fmoc-deblocking could be significantly diminished when nonpolar dichloromethane was used as solvent (0.5% after 4 h). Under these conditions the reaction was slow (Figure 4), but could be accelerated if 1 equiv of DIEA was added. However, the most efficient method of performing such very hindered couplings involved prior treatment with a silylating agent[58] which led to a clear increase in the rate of formation of Fmoc-NMeAib-Aib-OMe, with almost no dibenzofulvene being observed (0.3% after 4 h).

In Situ Generation and Utilization of Protected Amino Acid Fluorides

While it is important that most protected amino acid fluorides can be isolated and purified prior to their use, it would be equally valuable, especially for applications to continuous stepwise syntheses, to be able to take advantage of the special properties of these highly reactive coupling reagents without their actual isolation. This is now possible following the development of a new class of coupling reagents, the fluoroformamidinium salts. Thus, tetramethylfluoroformamidinium hexafluorophosphate (TFFH, **10**) can be synthesized by treatment of the readily available chloroformamidinium salt **9** with an excess of dry KF in acetonitrile (eq 4).[61] The properties of TFFH mark it as a very promising reagent: inexpensive, easily handled, and nonhygroscopic. Treatment with a protected amino acid in the presence of a tertiary



eq. 4

9 **10**

amine yields the corresponding protected amino acid fluoride which may be isolated if desired. Thus, TFFH represents an alternative to cyanuric fluoride and DAST for the preparation of stable acid fluorides. On the other hand, under appropriate conditions, TFFH can also act as a direct coupling reagent. As an example, pentapeptide **11**, H-Tyr-Aib-Aib-Phe-Leu-NH_2, a system which is not readily assembled via standard solid phase protocols, can be obtained in 88% yield (92% pure by HPLC analysis) via TFFH. The results are thus comparable to those obtained with preformed acid fluorides. TFFH has also been successfully used in the few cases (*e.g.*, His, Arg) where a shelf-stable, preformed protected amino acid fluoride is not yet available.[61]

For rapid solution phase peptide assembly, TFFH can be added directly to the one- or two-phase coupling medium in place of the preformed acid fluoride. No modification in the subsequent workup procedure is required (TAEA deblocking, buffer extraction, etc.). The pentapeptide leucine enkephalin (H-Tyr-Phe-Gly-Gly-Leu-OH) was assembled by this fast Fmoc/TAEA/TFFH technique in a yield of 48% (purity according to HPLC analysis 93%).

Concluding Remarks

Studies outlined in this Account have established that Fmoc-amino acid fluorides, whether as the stable, isolated species or as intermediates generated *in situ*, represent convenient inexpensive reagents for peptide coupling. Rapid coupling occurs even for sterically hindered amino acids.[62] In the case of preformed acid fluorides, coupling does not require the presence of base, thus precluding the incursion of any base-catalyzed side reactions, including loss of configuration at the carboxyl group undergoing reaction. Although more restricted in their applicability, the corresponding acid chlorides may sometimes be advantageous.[64]

We thank the National Science Foundation and the National Institutes of Health for their long-term support of this work at Amherst and the Deutsche Forschungsgemeinshaft and the Fonds der Chemischen Industrie for the work in Berlin. This work would not have been possible without the dedicated effort of our co-workers whose names are cited in the references. The library facilities of the Technische Universität and Freie Universität, Berlin, were invaluable in probing the early literature during the final preparation of this Account.

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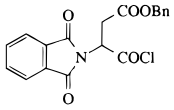
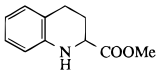
(62) For an example of the use of protected amino acid fluorides to couple cleanly in a case where other coupling agents [DCC/DMAP, DCC/HOBt, isobutyl chloroformate and bis(2-oxo-3-oxazolidin-*N*-yl)phosphinic chloride (BOP-Cl)[63]] gave significant loss of configuration, see: Marsden, S. P.; Depew, K. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1994**, *116*, 11143. In this case the acid fluoride was generated from cyanuric fluoride in the presence of pyridine and two-phase coupling carried out in the presence of $NaHCO_3$.

(63) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342.

(64) A recent example involved coupling of acid chloride **i** with amino acid ester **ii**, a reaction which failed with the corresponding acid fluoride.



i



ii

With either $NaHCO_3$ or 2,6-di-*tert*-butylpyridine as hydrogen chloride acceptor, reaction occurred with at least 99.3% diastereomeric purity whereas in the presence of *N*-methylmorpholine a 50:50 mixture of the diastereomers resulted. See: Robl, J. A.; Karanewsky, D. S.; Asaad, M. M. *Tetrahedron Lett.* **1995**, *36*, 1593.