ORGANIC LETTERS

2003
Vol. 5, No. 7
975−977

Complex Polyfluoride Additives in Fmoc-Amino Acid Fluoride Coupling Processes. Enhanced Reactivity and Avoidance of Stereomutation†

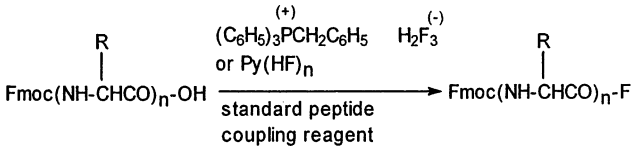
Louis A. Carpino,*,‡ Dumitru Ionescu,‡,§ Ayman El-Faham,‡,|| Michael Beyermann,*,⊥ Peter Henklein,*,# Christiane Hanay,# Holger Wenschuh,▽ and Michael Bienert⊥

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, Institute of Molecular Pharmacology, Robert-Rössle-Str. 10, 13125 Berlin, Germany, Institut für Biochemie, Humboldt-Universität, Monbijoustr. 2, 10117 Berlin, Germany, and Jerini AG, Invalidenstr. 130, 10115 Berlin, Germany

carpino@chem.umass.edu; beyermann@fmp-berlin.de; peter.henklein@charite.de

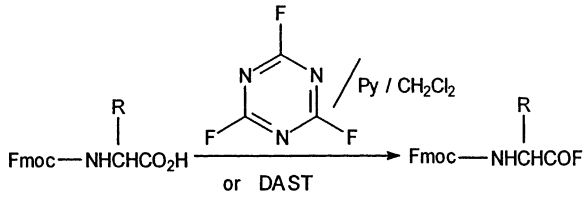
Received November 18, 2002

ABSTRACT



Isolated Fmoc amino acid fluorides have previously been shown to be among the most efficient reagents for peptide bond formation. Now, it has been found that anionic, polyhydrogen fluoride additives are capable of diverting many of the classical peptide coupling processes to acid fluoride couplings. Examples include the use of N-HBTU or N-HATU and the carbodiimide technique. As HF-containing species, these additives provide a more suitable medium for the coupling of systems that are sensitive to loss of configuration at the reactive carboxyl function.

Previously,[1] it has been shown that protected amino acid fluorides can be synthesized (eq 1) and used as convenient, highly efficient reagents for both solution and solid-phase syntheses.



Relative to the use of other reagents for effecting peptide coupling readily or alternatively without loss of configuration at the carboxylic acid residue, the acid fluorides are advantageous in the synthesis of systems that incorporate hindered

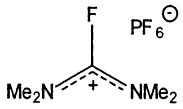
† Abbreviations used: ACP = acyl carrier protein decapeptide (64−75); Aib = α-aminoisoburyric acid; BOP = benzotriazol-1-yl-*N*-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate; PTF = benzyltriphenylphosphonium dihydrogen trifluoride; BSA = *N*,*O*-bis(trimethylsilyl)acetamide; DAST = (diethylamino)sulfur trifluoride; DCC = dicyclohexylcarbodiimide; DCM = dichloromethane; DIC = diisopropylcarbodiimide; DIEA = *N*,*N*-diisopropylethylamine; DMF = *N*,*N*-dimethylformamide; N-HATU = 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-b]pyridinium hexafluorophosphate 3-oxide; N-HBTU = 1-[bis(dimethylamino)methylene]-1-*H*-benzotriazolium hexafluorophosphate 3-oxide; N-HAPyU = 1-(1-pyrrolidinyl-1-*H*-1,2,3-triazolo[4,5-b]pyridinylmethylene)-pyrrolidinium hexafluorophosphate 3-oxide; pNA = *p*-nitroanilide; PAL-PEG-PS = peptide amide linker (5-(4-aminomethyl)-3,5-dimethoxyphenoxy)-valeric acid) on poly(ethyleneglycol)/polystyrene support (Applied Biosystems); Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; PyAOP = 7-azabenzotriazol-1-yloxytris-(pyrrolidino)phosphonium hexafluorophosphate; PyBroP = bromotris(pyrrolidino)phosphonium hexafluorophosphate; N-TBTU = 1-[bis(dimethylamino)methylene]-1-*H*-benzotriazolium tetrafluoroborate 3-oxide; TFFH = tetramethylfluoroformamidinium hexafluorophosphate; TFA = trifluoroacetic acid; TG-S RAM = tentagel Rink amide resin (Rapp Polymere); Trt = trityl.

‡ University of Massachusetts.
§ On leave from the Department of Chemistry, Faculty of Chemistry, University of Bucharest, 70346 Bucharest, Romania.
|| On leave from the department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt.
⊥ Institute of Molecular Pharmacology.
Humboldt-Universität.
▽ Jerini AG.

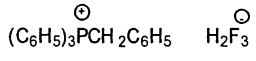
10.1021/ol020235a CCC: $25.00
Published on Web 03/05/2003

amino acids such as *a*-aminoisobutyric acids or N-substituted amino acids, respectively.[1d] In comparison with amino acid chlorides, which are generally more reactive than the acid fluorides, the latter are unique in several ways: (a) *tert*-butyl and trityl side chain protecting groups can be accommodated; (b) conversion to the corresponding oxazolone in the presence of tertiary organic bases does not occur, thus minimizing the danger of stereomutation; and (c) coupling reactions occur readily in the complete absence of an organic base, again avoiding possible loss of configuration. In view of these properties, the acid fluorides resemble activated esters more than acid chlorides or acid bromides. In employing Fmoc amino acid fluorides in practical peptide synthesis, difficulties were encountered only in the case of two amino acids: histidine and arginine. In the former case, while Fmoc-His(Trt)-F has been synthesized and used in coupling reactions, its long-term stability is in doubt. For sulfonamide-protected arginine derivatives, e.g., Fmoc-Arg-(Pbf)-OH, the corresponding acid fluorides could not be synthesized due to their facile cyclization to the corresponding lactam.

Recently the fluoroformamidinium salt TFFH **3** has been shown to act as a coupling reagent that proceeds via in situ conversion to an acid fluoride.[2]



3



4

Reagent **3** can be handled in air in the same way as the common onium reagents[3] such as N-HATU[4] and N-XBTU (X = H, T)[5] by base-catalyzed activation. For some amino acids, e.g., Fmoc-Aib-OH, it was found that the use of TFFH gave results that were less satisfactory than those obtained with isolated amino acid fluorides. The deficiency was traced to inefficient conversion to the acid fluoride, which under the conditions used (2 equiv of DIEA) was accompanied by the corresponding symmetric anhydride and oxazolone. On the other hand it is now shown that if a fluoride additive such as **4** (PTF) is present during the activation step, the latter two products are avoided and a maximum yield of acid fluoride is obtained. Assembly of the difficult pentapeptide **5** via TFFH coupling in the presence of **4** gave a product of quality similar to that obtained via isolated acid fluorides (Supporting Information).

More interestingly, conversion of the acid to the acid fluoride was also observed via treatment with N-HATU or N-HBTU in the presence of additive PTF.[6] In this way excellent syntheses of difficult peptides could be achieved in cases where N-HBTU itself gave poor results due to the sluggish reactivity of OBt esters. Examples include the syntheses of **5**, Aib[67,68]ACP(65−74)amide **6**, and alamethicin amide **7**[7] for all of which the products obtained with and without PTF additive are compared in Figures 1, 3, 4, and 5 (Supporting Information).

H-Tyr-Aib-Aib-Phe-Leu-NH_2

5

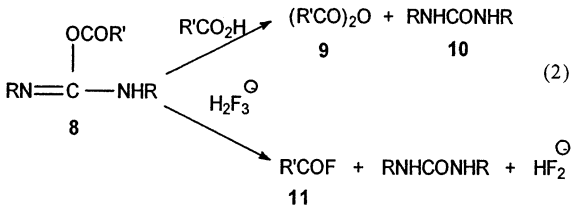
H-Val-Gln-Aib-Aib-Ile-Asp-Try-Ile-Asn-Gly-NH_2

6

Ac-Aib-Pro-Aib-Ala-Aib-Ala-Glu-Aib-Val-Aib-
Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phe-NH_2

7

By this means, the relatively inexpensive reagents N-HBTU or N-TBTU provide peptides of equal or greater quality than those obtained via N-HATU or isolated acid fluorides. Regardless of which coupling reagents are used in these reactions, a tertiary base such as DIEA is required in the activation step. At least in the case of systems that undergo facile loss of configuration, the presence of a base may be deleterious and at any rate is not required for the actual coupling step in the case of acid fluorides.[8] It is thus particularly significant that acid fluorides can be generated *in the absence of base* via carbodiimides.[9] The carbodiimide method of peptide activation is believed to involve transient formation of a labile *O*-acylisourea **8**, which in the presence of a second equivalent of carboxylic acid is converted to the symmetric anhydride **9**, which represents the active coupling species.[10] Now it has been found that in the presence of PTF and DCC or DIC, the putative *O*-acylisourea intermediate is diverted to the acid fluoride.



8 **9** **10** **11** (2)

Under optimum conditions, in a nonpolar solvent such as DCM, the activation is rapid, thus serving to guarantee an

(1) (a) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H.-G.; DeSelms, R. H. *J. Am. Chem. Soc*. **1990**, *112*, 9651. (b) Carpino. L. A.; Mansour, E. M. E.; Sadat-Aalaee, D. *J. Org. Chem*. **1991**, *56*, 2611. (c) Kaduk, C.; Wenschuh, H.; Beyermann, M.; Forner, K.; Carpino. L. A.; Bienert, M. *Lett. Pept. Sci*. **1995**, *2*, 285. (d) Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.* **1996**, *29* (6), 268.

(2) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc*. **1995**, *117*, 5401.

(3) (a) Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem., Int. Ed*. **2002**, *41*, 441. (b) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem Commun*. **1994**, 201.

(4) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(5) (a) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *Synthesis* **1984**, 572. (b) Knorr, R.; Treciak, A.; Bannworth, W.; Gilllessen, D. *Tetrahedron Lett.* **1989**, 1927.

(6) Infrared studies showed that under the conditions used, preactivation of a protected acid with N-HATU or N-HBTU in the presence of **4** gave a mixture of the acid fluoride and the OAt or OBt ester, respectively. However, conversion to the acid fluoride was never complete.

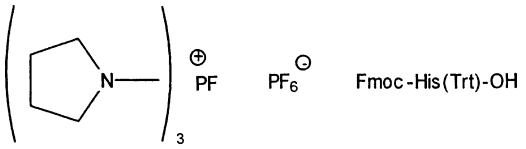
(7) For the first solid-phase syntheses of these hindered sequences via isolated Fmoc amino acid fluorides, see: Wenschuh, H.; Beyermann, M.; Krause, E.; Brudel, M.; Winter, R.; Schümann, M.; Carpino, L. A.; Bienert, M. *J. Org. Chem*. **1994**, *59*, 3275.

(8) Wenschuh, H.; Beyermann, M.; El-Faham, A.; Ghassemi, S.; Carpino, L. A.; Bienert, M. *J. Chem. Soc., Chem. Commun*. **1995**, 669.

(9) The recent appearance of a paper by Chen and co-workers (*J. Fluor. Chem*. **2002**, *115*, 75) on the generation of acid fluorides via DCC/Py-$(HF)_n$ has prompted us to record our results in this area.

efficient coupling process. As is the case for the usual carbodiimide activation process,[11] reaction is slow in DMF. Thus for assembly of **6**, activation was carried out in DCM via DIC/PTF, the solvent removed and coupling completed in DMF. An excellent synthesis of **6** resulted, whereas normal DIC methodology in the absence of PTF gave only the des-Aib nonapeptide.

It is to be expected that the fluoride additive will be of general utility for the improvement of most of the common coupling methodologies. We were, however, surprised to encounter at least one exception: coupling reagents such as BOP,[12] PyBrOP,[13] PyAOP,[3b] etc., which are built around phosphonium salt residues, are completely ineffective in the presence of PTF. It may be possible to rationalize such results on the basis of the exceptionally high strength of the P−F linkage.[14] Fluorophosphonium salt **12** was prepared from PyBroP by treatment with KF in acetonitrile. Treatment of **12** with Fmoc-Aib-OH in the presence of DIEA gave no acid fluoride. Similarly, attempted assembly of ACP via PyAOP in the presence of PTF gave no trace of peptide material.

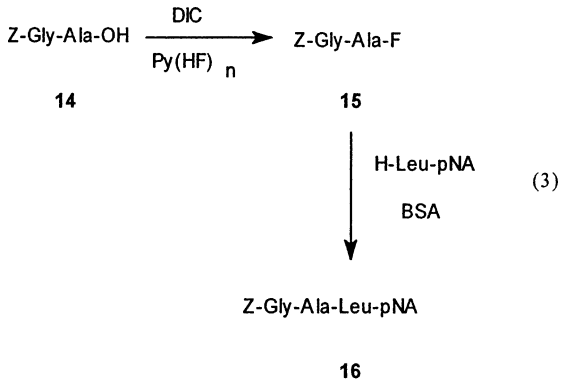


12 **13** Fmoc-His(Trt)-OH

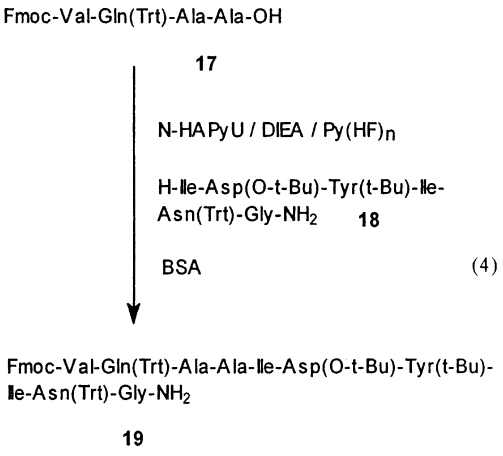
Because the fluoride additive binds excess hydrogen fluoride as part of the complex dihydrogen trifluoride anion, an accompanying acidic buffering effect might prove to be of value in the case of coupling reactions where loss of configuration at the activated carboxylic acid residue might be important. Such a protective effect was in fact observed in the case of the sensitive histidine derivative **13** upon reaction with proline amide, which with TFFH/DIEA under ordinary conditions gives the desired dipeptide in good yield with 7.4% stereomutation. In the presence of additive **4**, stereomutation drops to 1.8%.

A similar technique allowed the generation and utilization of peptide acid fluorides, although in this case the phosphonium salt **4** was substituted by the more acidic pyridine−hydrogen fluoride reagent.[15] Benoiton[16] has previously demonstrated the value of related peptide active esters. The increased reactivity of acid fluorides should allow enhancement of the segment coupling processes. With 5 equiv of $Py(HF)_n$ in place of **4**, dipeptide acid **14** gave with DIC model peptide acid fluoride **15**, which proved to be stable for several hours at room temperature.

Treatment of **15** with leucine *p*-nitroanilide in the presence of 1 equiv of bis(trimethylsilyl)acetamide (BSA)[17] gave the



tripeptide anilide **16** in 97% yield after 2 min with only 0.3% of the D-Ala stereoisomer. This compares with the 1% level of stereomutation observed in the absence of $Py(HF)_n$. The practical value of this technique was confirmed by its application to the [4 + 6] segment coupling leading to the protected ACP decapeptide ester **19**. In this case, the loss of configuration at Ala amounted to 0.5% as opposed to 1.5% when the reaction was carried out in the absence of $Py(HF)_n$. In both cases, the loss of configuration was determined by the method of Kusomoto et al.[18]



Acknowledgment. We are indebted to the National Science Foundation (NSF CHE-9003192) and the National Institutes of Health (GM-09706) for support of the work in Amherst. The work in Berlin was supported by the Deutsche Forschungsgemeinschaft He 2131/1-1.

Supporting Information Available: Experimental details for synthesis of additive **4** and representative solid-phase and solution syntheses, in its presence and absence, with complete characterization data for the derived peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

OL020235A

(10) Mikolajczyk, M.; Kielbasinski, P. *Tetrahedron* **1981**, *37*, 233.
(11) Beyermann, M.; Henklein, P.; Klose, A.; Sohr, R.; Bienert, M. *Int. J. Pept. Prot. Res.* **1991**, *37*, 252.
(12) Dormoy, J.-R.; Castro, B. *Tetrahedron Lett.* **1979**, 3321.
(13) Coste, J.; Frérot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437.
(14) Pimental, G. C.; Spratley, R. D. *Chemical Bonding Clarified Through Quantum Mechanics*; Holden-Day: San Francisco, 1969; p 311.
(15) Olah, G. O.; Welsh, J. T.; Vankar, Y. D.; Nojima, M.; Kerekes, I.; Olah, J. A. *J. Org. Chem.* **1979**, *44*, 3872.
(16) Benoiton, N. L.; Lee, Y. C.; Chen, F. M. F. In *Peptides 1994, Proc. of the 23rd European Peptide Symposium*; Maia, H. L. S., Ed.; Escom: Leiden, 1995; p 203.
(17) Wenschuh, H.; Beyermann, M.; Winter, R.; Bienert, M.; Ionescu, D.; Carpino, L. A. *Tetrahedron Lett.* **1996**, *37*, 5483.
(18) Kusomoto, S.; Matsukura, M.; Shiba, T. *Biopolymers* **1981**, *20*, 1869.