Tetramethylfluoroformamidinium Hexafluorophosphate: A Rapid-Acting Peptide Coupling Reagent for Solution and Solid Phase Peptide Synthesis

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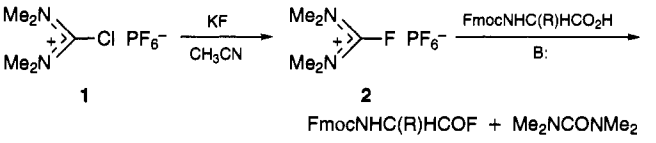
Recently Fmoc amino acid fluorides have been shown to be rapid-acting reagents for peptide coupling, useful for both solution and solid phase synthesis[1] and especially suited for the coupling of sterically hindered α,α-disubstituted amino acids, which are not easily handled by standard techniques.[2] Examples of the unique reactivity of these compounds include the first ever solid phase syntheses of the naturally-occurring peptaibols, peptide alcohols of about 20 units which are rich in such hindered amino acids.[3] For these syntheses, the Fmoc amino acid fluorides were isolated and purified by recrystallization prior to use. Now it has been possible to take advantage of the exceptional nature of amino acid fluorides without their isolation via utilization of a new reagent which effects clean *in situ* conversion of the acid to the fluoride under conditions similar to those which are now common in the case of uronium[4] and phosphonium[5] reagents derived from 1-hydroxybenzotriazole (HOBt)[6] and 1-hydroxy-7-azabenzotriazole (HOAt).[7] Of the 20-odd proteinogenic amino acids, only His and Arg could not be converted to shelf-stable amino acid fluorides for use in peptide assembly.[8ab] Now, however, generated as transient intermediates via TFFH, both can be routinely coupled in this form. In some cases for these two amino acids as well as for Asn,[8c,9] better results are obtained if 1 equiv of HOAt is present during the coupling process.

Tetramethylfluoroformamidinium hexafluorophosphate (TFFH,[10,11] **2**), a nonhygroscopic salt stable to handling under ordinary conditions, is obtained via reaction of tetramethylchloroformamidinium hexafluorophosphate (TCFH, **1**)[14] with excess anhydrous potassium fluoride.



1 **2**

FmocNHC(R)HCOF + $Me_2NCONMe_2$

3

Infrared examination shows that in the presence of *i*-Pr_2NEt (DIEA), Fmoc amino acids are converted via TFFH (ratio 2:1:1) to the acid fluorides **3**. In dichloromethane solution at room temperature, absorption at 1842 cm^{-1}, characteristic of the carbonyl fluoride unit, appears after about 3 min, with complete conversion to acid fluoride occurring after 8−15 min. For hindered amino acids [*e.g.*, α-aminoisobutyric acid (Aib)], complete conversion may require 1−2 h. If desired, the acid fluorides may be isolated and purified, making **2** a benign substitute for the corrosive reagent cyanuric fluoride. As an example of the preparative use of **2**, easily-racemized[15] Z-Phg-OH[16] gave optically pure Z-Phg-F in 69% yield. The optical purity of the product was established by reaction with proline amide in CH_2Cl_2 to give Z-Phg-Pro-NH_2,[3]contaminated by less than 0.1% of the DL-form.

TFFH appears to be an ideal coupling reagent for solid phase syntheses, being readily available, inexpensive, and capable of providing crude peptides of high quality. An example is the assembly of test peptide **4**, which, due to the difficult Aib-Aib

H−Tyr−Aib−Aib−Phe−Leu−NH_2

4

coupling, has previously[9] been used to demonstrate the superiority of HATU[16] over HBTU.[16] Using *N,N*-dimethylformamide (DMF) as solvent and a Biosearch 9050 instrument programmed for 7-min preactivation, 7-min deblocking, and 30-min coupling [5-fold excess of the acid, 10-fold excess of the base (DIEA)] for all amino acids except Aib-Aib, for which a 1-h double coupling was used, **4** was obtained in about 88% yield; purity of crude product, 92%; amount of des-Aib tetrapeptide, 4%. By contrast, under similar conditions the earlier syntheses[9] gave HATU, 94% purity, and HBTU, 43% purity. The somewhat less difficult sequence **4** having a Pro-Pro unit in place of Aib-

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(8) (a) Although Fmoc-His(Trt)-F has been synthesized and successfully used as a coupling reagent, its long-term shelf stability is questionable. (b) The activated forms of most common protected Fmoc arginine derivatives undergo more or less facile permanent deactivation via lactam formation. See: Juliano, L.; Juliano, M. A.; DeMiranda, A.; Tsuboi, S.; Okada, Y. *Chem. Pharm. Bull.* **1987**, *35*, 2550. (c) For reasons still not clear, the coupling of Fmoc-Asn(Trt)-OH via onium-type reagents is subject to some deficiencies.[9]

(9) Carpino, L. A.; El-Faham, A.; Minor. C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.

(10) All new compounds prepared during the course of this work showed consistent IR and NMR spectral data and elemental analyses for C, H, and N that agreed with theoretical values (± 0.3%).

(11) Preparation of TFFH: To a solution of 5.6 g of TCFH in 30 mL of dry CH_3CN was added 1.16 g of oven-dried anhydrous KF, and the mixture was stirred at room temperature for 2−3 h (^1H NMR monitoring). Longer times are required for large-scale preparations. Following filtration of KCl, the filtrate was evaporated and the residue recrystallized from CH_3CN−Et_2O to give 4.3 g (92.3%) of the nonhygroscopic fluoroformamidinium salt as white crystals: mp 111−112 °C; ^1H NMR ($CDCl_3$−DMSO-d_6) δ 3.175 (d, CH_3). Anal. Calcd for $C_5H_{12}F_7N_2P$: C, 22.72; H, 4.55; N, 10.61. Found: C, 22.73; H, 4.50; N, 10.63. Bis(tetramethylene)fluoroformamidinium hexafluorophosphate [BTFFH: mp 153−155 °C; yield 85.2%; ^1H NMR (CD_3CN) δ 2.03 (m, 4, CH_2), 3.84 (m, 4, CH_2N)] and 1,3-dimethyl-2-fluoro-4,5-dihydro-1*H*-imidazolium hexafluorophosphate [DFIH: mp 168−169 °C, ^1H NMR (CD_3CN) δ 2.9 (s, 6, CH_3), 3.88 (d, 4, CH_2)] were obtained similarly from bis(tetramethylene)chloroformamidinium hexafluorophosphate (BTCFH)[12] and 1,3-dimethyl-2-chloro-4,5-dihydro-1*H*-imidazolium hexafluorophosphate (DCIH).[13] Except for DFIH, which was somewhat moisture sensitive, these compounds were stable in air for weeks. A sample of TFFH left in an open beaker was examined by ^1H NMR for the corresponding urea and showed 1−2% hydrolysis after 2 weeks and 5−10% hydrolysis after 2−3 months.

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(16) Abbreviations not cited in the text: Phg, α-phenylglycine; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide;[17] HBTU, *N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide;[17] TAEA, tris(2-aminoethyl)amine; NMM, *N*-methylmorpholine; PS, 1,8-bis(dimethylamino)naphthalene; TMP, 2,4,6-collidine; TBFH, tetramethylbromoformamidinium hexafluorophosphate.

(17) For X-ray crystal data which correct previous solid state structural assignments to HATU and HBTU, see: Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. F.; Kates, S. A. *Lett. Pept. Sci.* **1994**, *1*, 57.

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Aib was obtained under similar conditions in 78.1% yield, purity 97.6%. Other standard peptide models were obtained similarly using 4−8 equiv of acid and 30-min coupling times: ACP[18] (87%, purity 92%), prothrombin[1a] (75%, purity 95%), and magainin II amide[19] (72%, purity 82%). Chloroformamidinium salt **1** and the corresponding bromo analog lack the general applicability of **2** as coupling reagents for solid phase syntheses.[20,24] The fluoroformamidinium reagent **2** could also be used as a direct substitute for the preformed acid fluorides in the Fmoc/TAEA[16] rapid solution synthesis of short peptide segments[1a,25] simply by adding the reagent to the two-phase mixture.

Finally, TFFH also proved useful for segment condensations providing 1 equiv of HOAt was present in order to preclude extensive epimerization.[26] Results obtained under these conditions were similar to those observed when HATU was used as the coupling reagent.[7,27] Thus for the coupling of Z-Phe-Val-OH to H-Ala-OMe·HCl in DMF via TFFH alone in the presence of DIEA, NMM,[16] PS,[16] or TMP,[16] the extent of formation of the LDL-isomer was about 25%, 23%, 8%, or 6%, respectively. On the other hand, for the same coupling carried out using TFFH/HOAt/DIEA or TFFH/HOAt/TMP, the amount of LDL-form was 2.0% or <0.1%, respectively. The last-named result agrees with that observed for coupling via HATU/TMP (<0.1% LDL-form). The massive extent of epimerization observed in the absence of additive may be related to the formation of oxazolone intermediates. Although it is an important property of urethane-protected amino acid fluorides to resist conversion to oxazolones in the presence of tertiary organic amines, this appears not to be the case for peptide segments.[28]

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Supplementary Material Available: Experimental procedures for the synthesis and use of TFFH (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(20) Previously the effect of BTCFH and DCIH on Fmoc amino acids has been reported to lead to the formation of mixtures of symmetric anhydrides and oxazolones.[13,21] Analogous results were described for the corresponding phosphonium analogs, bromotris(dimethylamino)phosphonium hexafluorophosphate (BroP),[22] bromotris(pyrrolidino)phosphonium hexafluorophosphate,[23] and chlorotris(pyrrolidino)phosphonium hexafluorophosphate.[23] In contrast to TFFH, fluorotris(pyrrolidino)phosphonium hexafluorophosphate (PyFloP)[10] does not give the amino acid fluoride or any other acylating species by reaction with a protected amino acid anion. The mechanistic implications of these differences between the fluoroformamidinium and fluorophosphonium salts are under examination. In our work, the chloro- and bromoformamidinium salts led to the formation of acid halides which could be isolated if desired. These differences account for the variation among the haloformamidinium salts as solid phase coupling reagents and the superiority of the fluoro derivatives. Thus, for the acylation of H-Ile-PEG-PS (loading 0.17 mmol/g) in DMF solvent via 5 equiv of Fmoc-Val-OH and 10 equiv of DIEA, the extent of acylation as measured by UV analysis was as follows:

	extent of coupling (%)		
time (min)	TFFH	TCFH	TBFH[16]
2	77	65	60
4	90	70	65
10	100	86	79

For rapid solid phase syntheses it is critical to use a base of sufficient strength to effect quick activation. TMP[16] by itself is too sluggish (coupling as above at 2, 4, and 10 min: 20, 26, and 32%, respectively), although a 1:1 mixture of TMP and DIEA is nearly as effective as DIEA alone (68, 80, and 100%). For the automated solid phase syntheses carried out here (DMF solvent) a Biosearch 9050 instrument was used, and the normal 7-min preactivation period of the standard protocol proved adequate in the case of DIEA or DIEA/TMP activation. On the other hand, for segment coupling, TMP alone or DIEA/TMP (1:1) is preferred in order to minimize loss of configuration.

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(24) In spite of their utility in effecting low-racemization coupling of N-methylated amino acids, these compounds proved unsuitable for solid phase syntheses. See: Roux, F.; Coste, J.; Frérot, E.; LeNguyen, D.; Jouin, P.; Loffet, A. *Peptides. Chemistry and Biology*; Proceedings of the 12th American Peptide Symposium; Smith, J. A., Rivier, J. E., Eds.; ESCOM: Leiden, 1992; p 625.

(25) Carpino, L. A.; Sadat-Aalaee, D.; Beyermann, M. *J. Org. Chem.* **1990**, *55*, 1673. As an example, leucine enkephalin was assembled in CH_2Cl_2/5% Na_2CO_3 using a 1.5 equiv excess of TFFH, 1-h couplings, and 15-min deblockings (TAEA). Yield of deblocked peptide, 48%; purity, 93% (HPLC).

(26) Akaji, Kuriyama, and Kiso have made use of HOAt as additive in connection with the use of DCIH as a coupling reagent for hindered systems. See ref 13.

(27) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695.

(28) The segment-like amino acid derivative *N*-benzoylphenylalanine is readily converted by TFFH/DIEA to a solid, mp 82 °C, believed to be the corresponding oxazolone on the basis of its IR[29] (KBr) (1820 and 1657 cm^{-1}) and ^1H NMR spectra. TFFH coupling of *N*-benzoylphenylalanine with alanine methyl ester in CH_2Cl_2 in the presence of DIEA or NMM led to fully racemic Bz-Phe-Ala-OMe (^1H NMR analysis) in 80% yield. The crude product exhibited infrared absorption at 1817 cm^{-1} due to contamination by the oxazolone.

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