Bis(tetramethylene)fluoroformamidinium Hexafluorophosphate(BTFFH): A Convenient Coupling Reagent for Solid Phase Peptide Synthesis

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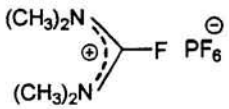
The onium reagent BTFFH has been shown to be a convenient reagent for the solid phase synthesis of a range of peptides incorporating sensitive amino acids.

In a preliminary publication a brief announcement was presented of the formation of acid fluorides by treatment of the acid with TFFH **1** or BTFFH **2**.[1] Because the reaction conditions for in situ generation of acid fluorides are compatible with normal protocols for peptide synthesis, TFFH **1** and BTFFH **2** are unique coupling reagents which take advantage of the exceptional properties of Fmoc-amino acid fluorides [2] without their separate isolation.



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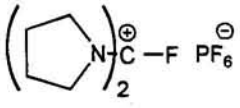

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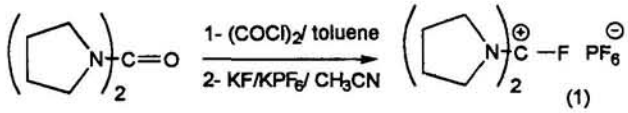
The present paper describes a new preparation of BTFFH modeled on an improved method for the synthesis of TFFH [3,4] (Eq. 1).



(1)

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Fluoroformamidinium salt **2** was shown to be as effective as isolated acid fluorides in either solution or solid phase peptide assembly even in the case of the two amino acids arginine and histidine, for which the preformed amino acid fluorides are not of long-term shelf stability. The reaction between Fmoc-Arg(Pbf)-OH, BTFFH and DIEA (1/1/2) in DMF was monitored by infrared analysis. The acid fluoride (1845 cm^{-1}) was generated within 2 min and although it cyclized slowly to the corresponding lactam (1794 cm^{-1}), a significant amount of the acid fluoride remained unreacted even after 60 min.

In order to confirm its efficiency in solid phase synthesis several peptides were assembled by means of BTFFH, including some which contained histidine and/or arginine. These included magainin I amide[5] and bradykinin[6] (Table 1 and Figures 1 and 2). The table lists other models made in the same way. In addition to its ease of synthesis and good shelf stability the use of BTFFH is advantageous over TFFH in view of the lack of formation of volatile or toxic by-products.[7]

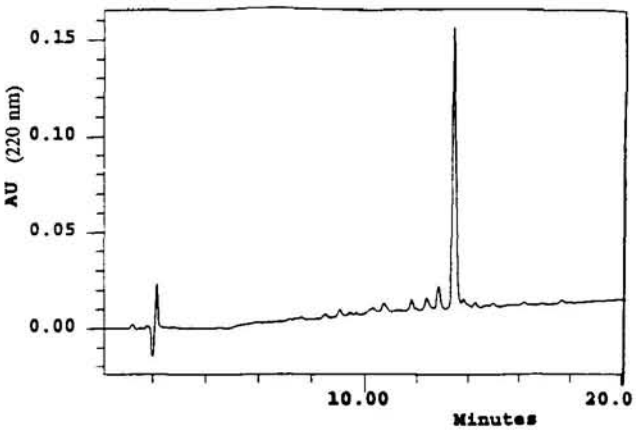

Figure 1. HPLC analysis of the crude sample of magainin I amide using a reversed phase Waters C_{18} Nova Pak column (4 μ, 60 °A, 3.9 x 150 mm) with a linear gradient of CH_3CN 25/55 (25 min) in H_2O containing 0.1 % TFA at a flow rate of 1 ml/min.

Table 1. Solid phase assembly of model peptides via BTFFH[a]

Name, Sequence	Yield, %	Purity,[b] %	MS / MALDI-TOF [c] Analysis Calcd $(M+H)^+$	Found
Magainin I amide, H-GIGKFLHSAGKFGKAGEIMKS-NH_2	78	84	2409.9	2410.3 $(M+H)^+$
Bradykinin amide, H-RPPGFSPFR-NH_2	83	86	1060.2	1060.5 $(M+H)^+$
ACP (65-74),[8] H-VQAAIDYING-NH_2	85	90	1063.2	1086.1 $(M+Na)^+$
Prothrombin amide,[9] H-ANKGFLEEV-NH_2	75	95	1006.2	1006.4 $(M+H)^+$
Human preproenkephalin (100-111),[10] H-YGGFMKRYGGFM-NH_2	78	89	1413.7	1436.3 $(M+Na)^+$
Insulin B-Chain (19-25),[6] H-CGERGFF-NH_2	83	89	1348.7	1371.2 $(M+Na)^+$
Substance P,[6] H-RPKQQFFGLM-NH2	80	90	814. 9	815.2 $(M+H)^+$

[a]All peptides were synthesized manually on a PAL-PEG-PS resin (0.18 mmol/g) (Perseptive Biosystems Inc.) in a plastic syringe attached to a vacuum manifold. The protocol involved treatment with 20 % piperidine in DMF for 10 min to remove the Fmoc group, 7-min preactivation of Fmoc-AA-OH/ BTFFH (5-fold excess) with a 10-fold excess of DIEA in DMF and a 30-min single coupling. [b]Purities are given by the percentage of the main peak relative to all other peaks present. In each case, in addition to the MS data given, all peptides were confirmed by peak overlap in the presence of an authentic sample. [c]All mass spectral analyses were carried out on a Perseptive Biosystems Voyager DE type MALDI-TOF instrument using sinapinic acid as matrix.

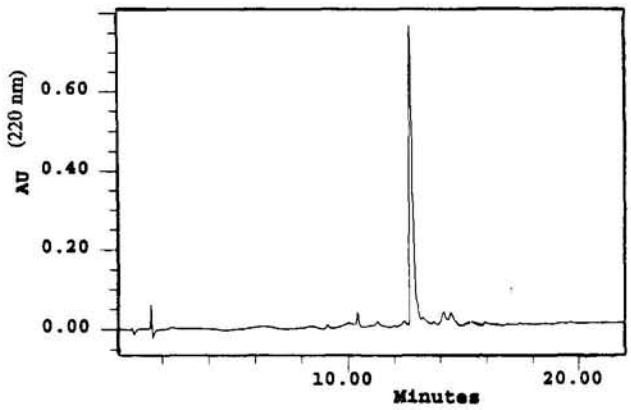

Figure 2. HPLC analysis of the crude sample of bradykinin using a reversed phase Waters C_{18} Nova Pak column (4 μ, 60 °A, 3.9 x 150 mm) with a linear gradient of CH_3CN 10/70 (30 min) in H_2O containing 0.1 % TFA at a flow rate of 1 ml/min.

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References and Notes

Abbreviations used for amino acids follow the recommendations of the IUPAC-IUB Commission and Biochemical Nomenclature [*J. Biol. Chem.*, **247**, 997 (1971)]. Other abbreviations are as follow: BTFFH = bis(tetra methylene)fluoroformamidinium hexafluorophosphate, DIEA = diisopropylethylamine, TFFH = tetramethylfluoroformamidinium hexafluorophosphate, DMF = dimethylformamide, Fmoc-PAL-PEG-PS = 5-[4-(9-fluorenemethyloxycarbonyl) aminomethyl-3,5-dimethoxyphenoxyl]valeroyl-PEG-PS.[11]

† This work was carried out at the Chemistry Department, University of Massachusetts, Amherst MA.

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4 Synthesis of BTFFH **2** : In a 1-L three-necked round bottomed flask equipped with a mechanical stirrer, addition funnel and reflux condenser, oxalyl chloride (17.5 ml, 0.2 mol) was added over a period of 10 min to a solution of bis(tetramethylene)urea (25.2 g, 0.15 mol) in dry toluene (200 ml) with vigorous stirring. The reaction mixture was refluxed for 3 h and then anhydrous ether was added (100 ml). The crude precipitate was washed with anhydrous ether (2 x 250 ml) and treated with a pre-dried mixture of KF (11.6 g, 0.2 mol) and KPF_6 (37.6 g, 0.2 mol) in dry acetonitrile (300 ml) . The reaction mixture was stirred at 60 °C for 3 h and then cooled to room temperature, filtered and washed with acetonitrile (3 x 20 ml). The combined filtrates were evaporated and the resulting oily residue was taken up in hot dry dichloromethane (200 ml). The cloudy solution was filtered while hot and concentrated under vacuum to approximately half its volume. Anhydrous ether was added with vigorous stirring to promote formation of a white crystalline solid. The precipitate was collected and dried under vacuum to give 42.6 g (90%) of the formamidinium fluoride as a white crystalline solid, mp 154 °C, ^{1}H NMR (CD_3CN) δ 2.03 (m, 4, CH_2), 3.84 (m, 4, CH_2N), (lit[1]: mp 153-5 °C, 85.2%).

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