

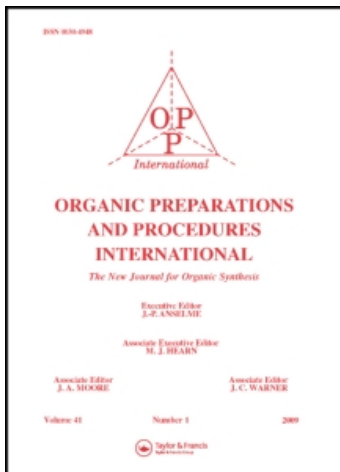
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### NEW SYNTHESSES OF

### *bis*(TETRAMETHYLENE)FLUOROFORMAMIDINIUM HEXAFLUOROPHOSPHATE (*BTFFH*) AND 1,3-DIMETHYL-2-FLUORO- 4,5-DIHYDRO-1H-IMIDAZOLIUM HEXAFLUOROPHOSPHATE (*DFIH*). UTILITY IN PEPTIDE COUPLING REACTIONS

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**NEW SYNTHESSES OF *bis*(TETRAMETHYLENE)FLUOROFORMAMIDINIUM  
HEXAFLUOROPHOSPHATE (BTFFH) AND 1, 3-DIMETHYL-2-FLUORO-4, 5-  
DIHYDRO-1H-IMIDAZOLIUM HEXAFLUOROPHOSPHATE (DFIH).**

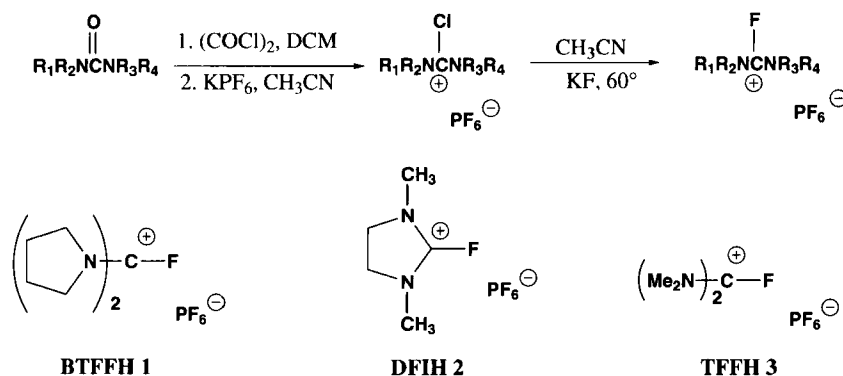
**UTILITY IN PEPTIDE COUPLING REACTIONS<sup>†</sup>**

Submitted by  
(05/05/98)

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In a preliminary publication, a brief announcement was given of the syntheses of BTFFH (1) and DFIH (2),<sup>1</sup> the method being modeled on a "wet" procedure previously devised for the synthesis of TFFH (3). More recently Vojkovsky and Drake<sup>2</sup> described in this Journal a simplified "dry" procedure for the synthesis of 3. Independently we developed a similar dry route to 3 and have now extended this method to both 1 and 2. These new syntheses are reported here with full experimental details. An alternative wet procedure for the synthesis of 3 has also been described.<sup>3</sup> Aside from its convenience and its potential application to large-scale syntheses, the superiority of the dry technique is especially clear in the case of DFIH. Indeed the previous method for the synthesis of 2 led to the isolation of material which was difficult to purify and in the semi-purified state showed some sensitivity to atmospheric moisture.



Relative to the corresponding chloro derivatives (TCFH, BTCFH and DCIH), the new fluoro analogs are advantageous for effecting peptide coupling, especially in the case of amino acids sensitive to loss of configuration at the C-terminal carboxylic acid residue. This difference holds even in the case where an additive such as HOAt<sup>4</sup> is used in order to minimize the loss of configuration (Table 1).

Infrared examination of the reaction mixture during the coupling process provides a possible rationale for these results. Activation of a protected amino acid by means of the chloro derivatives gives initially the oxazolone (IR: 1830 and 1685 cm<sup>-1</sup>) in the cases of TCFH, BTCFH and DCIH. In the presence of HOAt the oxazolone is converted to the OAt ester (IR: 1823 and 1723 cm<sup>-1</sup>) which is

less prone to suffer loss of configuration. For TFFH, BTFFH and DFIH, preactivation in the absence of HOAt gives initially the acid fluoride (IR: 1844  $\text{cm}^{-1}$ ) whereas if HOAt is present a mixture of acid fluoride and OAt ester is observed in the cases of TFFH and BTFFH, whereas in case of DFIH only the OAt ester is observed. Acid fluorides are less sensitive than acid chlorides to oxazolone formation and therefore to any of the various by-products which follow oxazolone formation.<sup>9</sup> For preformed HOAt-based coupling reagents such as HATU,<sup>4</sup> preactivation gives the OAt ester directly. If TFFH and HOAt are first mixed together rapid reaction occurs to give HATU, which then undergoes reaction in the same way as preformed HATU. The highest reactivity in the preactivation step (conversion of acid to OAt ester) is provided by DFIH but this does not necessarily make for cleaner reactivity as shown in Table 1.

**TABLE 1.** Effect of Haloformamidinium Salts on the Preservation of Configuration during Peptide Bond Formation in DMF in the Presence or Absence of HOAt<sup>a</sup>

Peptide	Coupling Reagent	Base	DL (%) <sup>b</sup>	
			Without Additive	With Additive (HOAt)
Z-Phg-Pro-NH <sub>2</sub>	TFFH	DIEA	7.4	1.7
	TCFH	DIEA	29.9	2.9
	BTFFH	DIEA	7.2	1.6
	BTCFH <sup>c</sup>	DIEA	29.8	2.8
	DFIH	DIEA	8.3	1.7
	DCIH <sup>d</sup>	DIEA	30.1	2.3
Z-Phe-Val-Pro-NH <sub>2</sub>	TFFH	TMP	—	2.7
	TCFH	TMP	—	3.8
	BTFFH	TMP	—	2.8
	BTCFH <sup>c</sup>	TMP	—	3.9
	DFIH	TMP	—	3.2
	DCIH <sup>d</sup>	TMP	—	8.3

- a) Standard protocol: test couplings were carried out by adding the coupling reagent (0.13 mmol) to a stirred and ice-cooled solution of the protected amino acid or protected dipeptide acid (0.125 mmol), H-Pro-NH<sub>2</sub> (0.125 mmol), and the chosen base (0.25 mmol) in 1 mL of DMF. After 1 h the ice bath was removed and stirring was continued for 3 h. For the reaction involving HOAt as additive (0.125 mmol) 0.33 mmol of base was used. The reaction mixture was diluted with 25 mL of EtOAc, and the organic layer was washed twice with 10 mL portions of 1 N HCl, twice with 10 mL portions of 10% NaHCO<sub>3</sub>, and twice with 10 mL portions of saturated NaCl solution. The organic solution was dried (MgSO<sub>4</sub>) and after filtration, the solvent was removed *in vacuo* at 30–35°. The resulting residue was dissolved in CH<sub>3</sub>CN for direct injection onto a C-18 column for HPLC analysis. The LL- and DL- forms of the test dipeptide have been described elsewhere,<sup>5</sup> as have the LLL- and LDL- forms of the test tripeptide.<sup>6</sup> b) The amount of racemization at Phg or epimerization at Val was measured by HPLC analysis as described in reference 6. c) Previously given the abbreviation PyCIU,<sup>7</sup> but modified here due to its relationship to the abbreviation chosen for the corresponding fluoro analog. d) Previously given the abbreviation CIP,<sup>8</sup> but modified here due to its relationship to the abbreviation chosen for the corresponding fluoro analog.

For the use of these reagents in peptide assembly by the solid phase technique the major differences were between the chloro and fluoro systems. Thus in the assembly of the difficult test sequence **4** the best results were obtained for BTFFH. The results are collected in Table 2.

**TABLE 2.** Solid Phase Assembly of Pentapeptide **4** (H-Tyr-Aib-Aib-Phe-Leu-NH<sub>2</sub>)<sup>a</sup>

Coupling Reagent	Pentapeptide <b>4</b> , % Without Additive	Pentapeptide <b>4</b> , % With Additive (HOAt)
TFFH	95.2	95.4
BTFFH	95.8	95.9
BTCFH	8.8	74.9
DCIH	9.1	72.7

a) In all cases the HPLC curves used to assess the purity of the pentapeptide consisted of only two peaks, that of the desired pentapeptide and that of the des-Aib tetrapeptide.

### EXPERIMENTAL SECTION

According to general techniques previously described;<sup>10</sup> analytical HPLC analyses were carried out with a Waters Nova Pak C<sub>18</sub> column (4μ, 60 Å, 3.9x150 mm). A Waters 996 Photodiode Array detector was used in all cases. Melting points were obtained using a MEL-Temp. II apparatus and are uncorrected. Mass spectral analyses were obtained on a Voyager DE type MALDI-TOF instrument (Perseptive Biosynthesis) using sinapinic acid as matrix.

**bis(Tetramethylene)fluoroformamidinium Hexafluorophosphate (BTFFH).**- In a 1 L three-necked round bottomed flask equipped with a mechanical stirrer, addition funnel and reflux condenser, oxalyl chloride (39 mL, 0.4 mol) was added over a period of 10 min to a solution of *bis*(tetramethylene)urea<sup>11</sup> (50.4 g, 0.3 mol) in dry methylene chloride (500 mL) with vigorous stirring. The reaction mixture was refluxed for 3 h and the solvent was removed under reduced pressure at 20-25°. The residue was washed with anhydrous ether (2x250 mL) and a pre-dried mixture of KF (23.2 g, 0.4 mol) and KPF<sub>6</sub> (75.2 g, 0.4 mol) was added. The resulting mixture was dissolved in 300 mL of dry acetonitrile and heated for 3 h at 60°. The reaction mixture was cooled to 20-25°, filtered and washed with acetonitrile (3x50 mL). The combined filtrates were evaporated and the resulting oily residue was taken up in hot methylene chloride and the cloudy solution was filtered while hot and concentrated under vacuum to approximately half the volume (200 mL). Anhydrous ether was added with vigorous stirring to promote precipitation of the salt as a white solid which was collected and dried under vacuum, mp. 154°, yield 91% (lit<sup>1</sup>. mp. 153-155°, 85%); <sup>1</sup>H NMR (CD<sub>3</sub>CN): δ 2.03 (m, 4, CH<sub>2</sub>), 3.84 (m, 4, CH<sub>2</sub>N).

*Anal.* Calcd for C<sub>9</sub>H<sub>16</sub>F<sub>7</sub>N<sub>2</sub>P: C, 34.17; H, 5.06; N, 8.86. Found: C, 34.29; H, 5.09; N, 8.76

The same method was applied to the synthesis of TFFH on a 1 mole scale to give the fluoro compound in 91% yield, mp. 110°. A similar procedure gave DFIH in a yield of 88%, mp. 168-169°.<sup>1</sup> In the latter case the <sup>1</sup>H NMR spectrum (CD<sub>3</sub>CN) [δ 2.9 (s, 6, CH<sub>3</sub>), 3.88 (d, 4, CH<sub>2</sub>)] gave no evidence for any of the starting material showing that complete conversion had occurred and the

fluoro compound as obtained by this method is relatively stable in air.

**Assembly of H-Tyr-Aib-Aib-Phe-Leu-NH<sub>2</sub>**<sup>12</sup>. - The pentapeptide was built up manually on an Fmoc-PAL-PEG-PS resin (0.18 mmol/g) using a plastic syringe which was attached to a vacuum manifold in order to effect rapid removal of reagents and solvents. For N- $\alpha$ -Fmoc removal piperidine-DMF (1:4) was used for 10 min. For coupling a 5-fold excess of Fmoc-amino acid and coupling reagent and a 10-fold excess of DIEA was used. The preactivation time was 7 minutes except when HOAt was present for which 1-2 min was used. For normal amino acids a 30-min single coupling was used whereas for the Aib-Aib unit double coupling was used. The pentapeptide was deblocked and removed from the resin by means of TFA/H<sub>2</sub>O (9:1) for 2 h at 20-25°. After filtration the TFA solution was concentrated under vacuum at 20-25°, and the crude peptide was precipitated by adding ether at 0°. The purity of the pentapeptide was determined by HPLC analysis using as mobile phase a linear gradient 5/95 CH<sub>3</sub>CN/H<sub>2</sub>O, 0.1% TFA in 25 min, flow rate 1 mL/min with detection at 220 nm (Table 2).

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- † Abbreviations used: TXFH = tetramethylhaloformamidinium hexafluorophosphate, BTXFH = bis(tetramethylene)haloformamidinium hexafluorophosphate, DXIH = 1,3-dimethyl-2-halo-4,5-dihydro-1H-imidazolium hexafluorophosphate, TMP = 2,4,6-trimethylpyridine (collidine), DMF = dimethylformamide, TFA = trifluoroacetic acid, DIEA = diisopropylethylamine, HOAt = 1-hydroxy-7-azabenzotriazole, DCM = dichloromethane, Phg =  $\alpha$ -phenylglycine.
- †† This work was carried out at the Chemistry Department, University of Massachusetts, Amherst MA 01003.
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dichloromethane was used for the preparation of BTCFH, yield 69%, mp. 148-149°, <sup>1</sup>H NMR (CD<sub>3</sub>CN): δ 2.06 (m, 4, CH<sub>2</sub>), 3.88 (m, 4, CH<sub>2</sub>N).

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## SYNTHESIS OF BENZO[c][2,7]NAPHTHYRIDINES AND 2,13-DIAZABENZO[b]CHRYSENE

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Over the past fifteen years marine organisms have furnished a number of biologically active natural products that contain the benzo[c][2,7]naphthyridine subunit.<sup>1</sup> In 1992 we reported a divergent synthesis of substituted benzo[c][2,7]naphthyridines that utilized an intramolecular pyridyne cyclization strategy.<sup>2</sup>