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1-Ethyl-2-Fluoro Pyridinium Salt: A Highly Efficient Coupling Reagents for Sterically Hindered Peptide Synthesis

Peng LI, Jie Cheng XU*

Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences,
Shanghai 200032

Abstract: 1-Ethyl-2-fluoropyridinium tetrafluoroborate (FEP) was shown to be a very efficient coupling reagent for the synthesis of the hindered peptide with fast reaction speed, low racemization and good yields.

Keywords: FEP, peptide, racemization, reactivity.

More and more attention for the incorporation of *N*-methyl or C_α,C_α-dialkyl amino acid residues into peptides has been paid in view of the increasing number of isolated naturally-occurring peptide bearing sterically hindered amino acid units, such as MeVal and Aib. However, the chain elongation of such peptide is often inefficient using conventional peptide coupling reagents, such as HOBt-based phosphonium[1], uronium[2] and immonium[3] type reagents. Recently, HOAt-derived onium salts[4] and halogenerated onium salts[5] were shown to be effective for the synthesis of peptide containing hindered amino acids, but the former are very expensive and not suitable for peptide synthesis in large scale; the latter usually result high racemization during coupling, especially for segment condensation. To tackle these problems, compound 1-ethyl-2-fluoro pyridinium tetrafluoroborate (FEP) was synthesized and successfully used for peptide synthesis with fast reaction speed, low racemization and good yields.

Reagent FEP could be easily prepared from 2-fluoropyridine by *N*-alkylation using triethyloxonium tetrafluoroborate in nearly quantitative yield[6]. The application of this reagent was evaluated using the model reaction (Z-Gly-Phe-OH + Val-OCH_3·HCl $\rightarrow$ Z-Gly-Phe-Val-OCH_3) by HPLC. **Table 1** and **Figure 1** showed that reagent FEP was much more reactive than widely used halogenerated reagents, such as PyBroP, PyClU, BTFFH and BOP-Cl. The coupling was completed within 2 minutes under the tested reaction conditions as shown in **Figure 1**. The coupling yield could be further improved if base was added slowly or dropwise to avoid reagent FEP reacted too violently. It is obvious that the racemization of product using FEP was much lower than using other halogenerated coupling reagents as shown in **Table 1**. If HOAt was added as additive, the racemization could be further suppressed.

Table 1 Comparison of racemization and reactivity of FEP with other coupling reagents[a]

Coupling Reagents	Yield% (t = 2 min)	$t^{1/2}$/min	D-isomer content%
PyClU	5.64	> 120	33.2
PyBroP	6.10	79	22.3
BOP-Cl[b]	5.34	~ 90	4.13
BTFFH	9.35	49	25.9
CMMM	2.31	> 120	31.4
CMBI	19.7	92	16.8
BEMT	45.9	—	2.72
FEP	76.5	< 2	2.33
FEP + HOAt	86.7	< 2	1.54

[a]Model reaction: Z-Gly-Phe-OH + Val-OCH_3·HCl → Z-Gly-Phe-Val-OCH_3. Reaction conditions: T - 10 °C, Base DIEA, Sol. CH_2Cl_2 (10 mL/mmol), substrate ratio: *N*-protected amino acid : amino acid ester hydrochloride : coupling reagent : Base = 1: 1.1: 1.1: 3.2 (mol). [b]Reagent BOP-Cl was not totally dissolved in CH_2Cl_2 at the beginning of the reaction, thus its $t^{1/2}$ value could not be evaluated accurately.

Figure 1 Comparison of reactivity of FEP with other coupling reagents

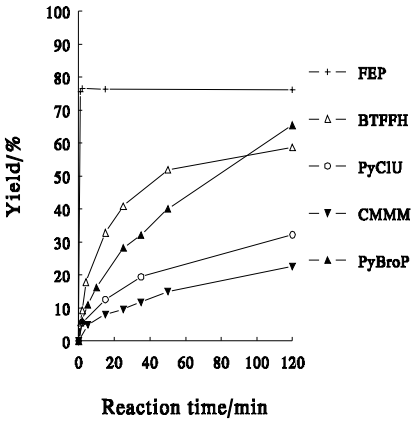


Table 2 Synthesis of oligopeptides using FEP as coupling reagent[a]

Peptide[a]	yield (%)[b]	mp (°C)	$[\alpha]_D$ (conc.,solv., temp.)
Boc-Val*-MeVal-OMe	92.1	oil	-136.0 (1, MeOH, 20 °C)
Boc-Pro*-Pro-OBzl	93.9	oil	- 127.4 (1, MeOH, 21 °C)
Z-Aib-Aib-OCH_3	95.3	108-110	—
Z-Leu*-Ala-OBu^t	88.7	glassy solid	- 38.2 (1, MeOH, 20 °C)
Fmoc-MeLeu*-MeVal-OCH_3	94.7	oil	-135.6 (1, MeOH, 20 °C)
Fmoc-Leu*-Ala-OBzl	91.0	124-126	- 47.9 (1, MeOH, 20 °C)
Z-Val*-Leu-Ala-OBu^t	96.7	131-132	- 56.3 (1, MeOH, 20 °C)

[a] The CO-NH bond formed in the peptide is indicated by *, all products were confirmed by ^1H NMR, EIMS and other characterizations. [b]Isolated yield based upon *N*-protected amino acid.

The efficiency of this pyridinium type reagent FEP was further approved by the successful synthesis of a series of oligopeptides, especially, the easy coupling between *N*-methyl amino acids. The reaction was carried out as that for reagent BEMT[7]. In a typical experimental procedure, DIEA (3.2 equiv.) was added slowly to a cooled mixture (-10 °C) of *N*-protected amino acid (1 equiv.), amino acid ester hydrochloride (1.1

equiv.), and FEP (1.1equiv.) in CH_2Cl_2 (2-4 mL/mmol), stirred for 1 minute under cold condition and then for an hour at room temperature; the reaction time should be properly prolonged for the coupling between *N*-methyl amino acids and could be monitored by TLC.

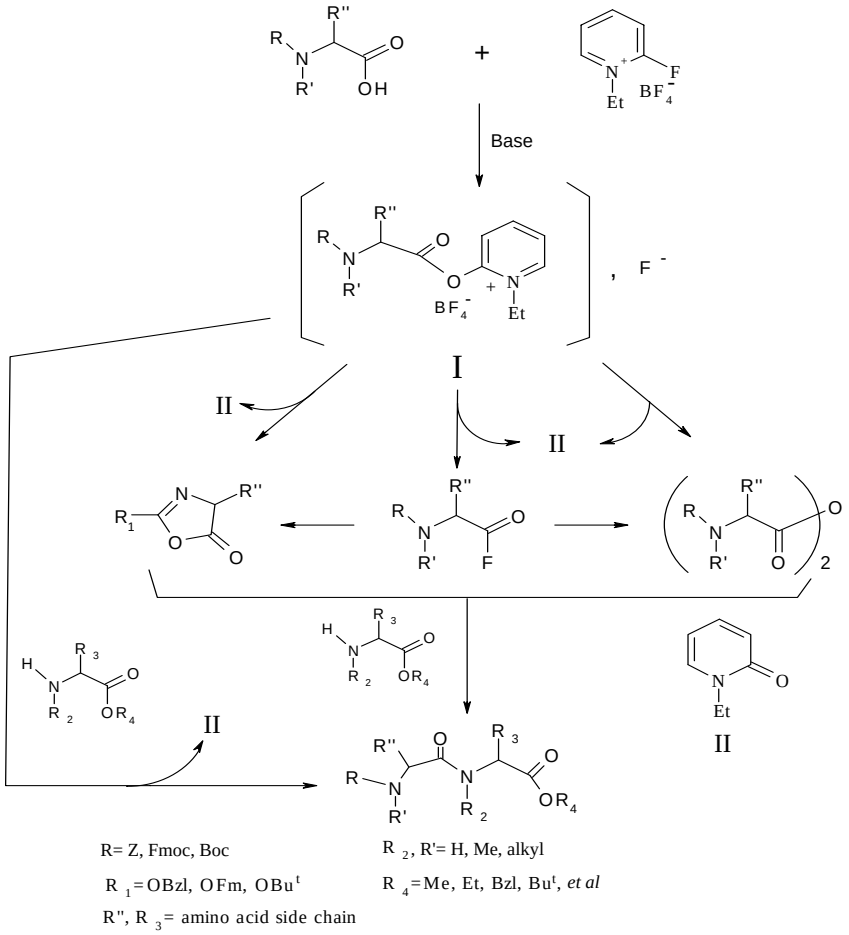
Reagent FEP could also be used to synthesize esters, especially the preparation of active esters such as benzotriazolyl esters, pentafluorophenyl esters and succinyl esters which were often used for the synthesis of lactone and lactams (**Table 3**).

Table 3 Synthesis of esters using FEP as coupling reagent

Substrate		Product[a]	Yield[b]	mp
Carboxylic acid	Alcohol		(%)	(°C)
Boc-Aib-OH	HOBt	Boc-Aib-OBt	81.5	oil
Boc-Aib-OH	KOPfp	Boc-Aib-OPfp	75.6	105-106
Fmoc-Sar-OH	HOSu	Fmoc-Sar-OSu	81.3	glassy solid
CBZ-Ala-OH	Bu^t-OH	CBZ-Ala-OBu^t	64.1	oil

[a]All products were confirmed by ^{1}H NMR, EIMS and other characterizations. [b]Isolated yield based upon *N*-protected amino acid.

Figure 2 The proposed reaction mechanism for coupling reagent FEP



To elucidate the mechanism of FEP mediated coupling reaction, we carried on the model reaction in $CDCl_3$ by treating Boc-Val-OH with FEP in the presence of NEt_3. It was speculated that the first step of the coupling was the formation of an unstable acyloxypyridinium salt **I**, which in turn reacted with the amino component to give the product or was competitively converted into the acid fluoride which subsequently converted into the dipeptide by aminolysis. ^{1}H NMR spectrum of the reaction mixture indicated that a small amount of the corresponding acid anhydride and 5(4*H*)-oxazolone was also formed from the acyloxypyridinium salt or acid bromide (**Figure 2**). The main byproduct of the coupling reaction was *N*-ethyl-2-pyridone **II** which could be isolated from the reaction mixture and characterized by ^{1}H-NMR, EI-MS and IR.[8] Comparing to halouronium and halophosphonium type coupling reagents, the high reactivity of FEP may attribute to the unstability of the pyridinium salt itself and the higher reactivity of the (acyloxy)pyridinium salt **I** than those of (acyloxy)uronium and (acyloxy)-phosphonium salts due to obvious electronic effect.

In conclusion, the pyridinium type coupling reagent FEP was shown to be of very high reactivity, low racemization and excellent yields for the peptide synthesis, especially for the acylation of *N*-methyl or C_α, C_α-dialkyl amino acids.

Acknowledgment

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References and notes

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6. Coupling reagent FEP was prepared from 2-fluoropyridine by alkylation using $Et_3O^+BF_4^-$ in $ClCH_2CH_2Cl$ at room temperature in 94.7% yield. mp 52-53 °C. ^{1}H NMR (300 MHz, d_6-acetone) δ 1.69 (t, J = 7.3 Hz, 3H), 4.85 (q, J = 7.3 Hz, 2H), 8.02-9.11 (m, 4H) ppm. ^{19}F NMR (60 MHz, d_6-acetone, CF_3COOH) δ 75.2 (s, 1F) ppm. IR (KBr) ν = 3039, 1643, 1587, 1519, 1475, 1307, 1070, 847, 786, 725, 522 cm^{-1}. FAB-MS *m/z* 126. Anal. Calcd. for $C_7H_9BF_5N$: C 39.48, H 4.26, N 6.58%. Found: C 39.32, H 4.17, N 6.54%.
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8. *N*-ethyl-2-pyridone: ^{1}H NMR (300 MHz, d_6-acetone) δ 1.26 (t, J = 7.1 Hz, 3H), 3.97 (q, J = 7.2 Hz, 2H), 6.22 (m, 1H), 6.41 (d, J = 9.3 Hz, 1H), 7.40 (m, 1H), 7.62 (dd, J_1 = 6.4 Hz, J_2 = 2.1 Hz, 1H) ppm. IR (KBr) ν = 3437, 2983, 1654, 1575, 1542, 1450, 1352, 1154, 1068, 771 cm^{-1}. EI-MS *m/z* 124 $[M + H]^+$, 123 $M^{+\cdot}$.

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