2-Bromo-1-ethyl Pyridinium Tetrafluoroborate (BEP): A Powerful Coupling Reagent for *N*-Methylated Peptide Synthesis

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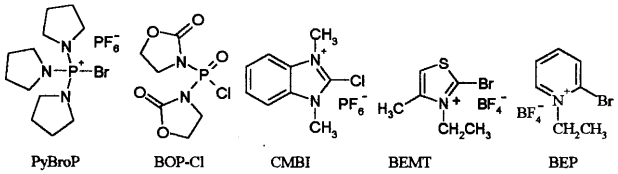
2-Bromo-1-ethyl pyridinium tetrafluoroborate (BEP) was firstly utilized to the synthesis of peptides containing *N*-methyl amino acid residues both in solution and solid phase, and demonstrated high reactivity, low racemization and excellent yields, which were approved by the successful synthesis of a series of oligopeptides and hindered peptide fragments, such as the 8-11 segment of Cyclosporine A and the pentapeptide moiety of Dolastatin 15.

Since the first phosphonium salt BOP[1] was exploited and used to peptide synthesis, many onium type coupling reagents have been designed, synthesized and widely used in peptide chemistry, such as HOBt-derived onium salts: HBTU,[2] PyBOP,[3] HBPyU,[4] HBPipU,[5] HBMDU[6] and the corresponding HOAt-based onium salts[7]: AOP, PyAOP, HATU, HAPyU, HAMDU. To further enhance the coupling efficiency during peptide synthesis, we have designed and synthesized immonium and thiazolium type coupling reagents, such as BOMI,[8a] BDMP,[8b] BPMP, AOMP and BEMT[8c] based upon our previous studies.[8d-8g] We now introduce pyridinium salt,[9a,9b] 2-bromo-1-ethyl pyridinium tetrafluoroborate (BEP), of which analogues 1-methyl-2-halopyridinium iodides were shown to be efficient for the syntheses of esters,[9c] lactones[9d] and carboxamides,[9e] into this field and demonstrate its high efficiency in peptide synthesis, especially in the incorporation of hindered *N*-methyl amino acids into peptides, such as cyclosporins,[10] didemins[11] and dolastatins.[12]

Comparing to other halogenerated coupling reagents PyClU,[13a] CIP,[13b] BOP-Cl[13c] and PyBroP,[13d] Pyridinium salt BEP can be more readily synthesized by the *N*-alkylation of 2-bromo pyridine using triethyloxonium tetrafluoroborate with nearly quantitative yield as colorless shelf-stable crystals.[14]



HPLC monitoring the model reactions (Z-Me-Val-OH + Me-Val-OMe·HCl→Z-Me-Val-Me-Val-OMe and Z-Gly-Phe-OH + Val-OCH_3·HCl → Z-Gly-Phe-Val-OCH_3) showed that reagent BEP behaved higher reactivity and lower racemization during coupling comparing to CBMI, PyBroP, PyClU and BTFFH et al. (Table 1 and Figure 1).

To further demonstrate the efficiency of BEP during peptide coupling, a series of oligopeptides were synthesized as shown in Table 2. We also synthesized successfully two extensively *N*-alkylated peptide segments. During the synthesis of 8-11 tetrapeptide fragment of cyclosporine A, the *C*-terminal was

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

Coupling reagent	Yield/% (t = 2 min)	D-isomer content/%
PyBroP	6.10	22.3
PyClU	5.64	33.2
BTFFH	9.35	25.9
BOP-Cl	5.34	4.13
CMBI	19.7	16.8
BEP	50.8	4.59
BEP + HOAt	75.9	1.42

[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, substrate ratio: *N*-protected amino acid : amino acid ester hydrochloride : coupling reagent : Base = 1: 1.1: 1.1: 3.2 (mol).

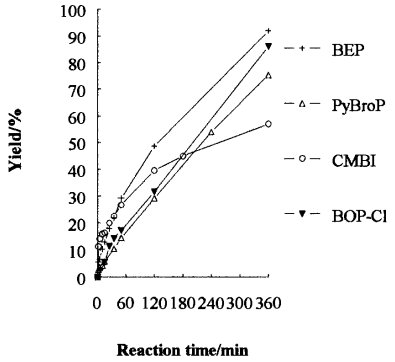


Figure 1. Comparison of reactivity of BEP with other coupling reagents[a].
[a]Model reaction: Z-MeVal-OH + MeVal-OCH_3·HCl → Z-MeVal-MeVal-OCH_3. Reaction conditions: T: 25 °C; Base: DIEA; Solvent: CH_2Cl_2 (0.1 M); Substrate ratio: *N*-protected amino acid : amino acid ester hydrochloride : Coupling reagent : Base = 1: 1.1: 1.1: 3.2 (mol).

Table 2. Preparation of peptides using BEP in solution[a]

Peptide[b]	yield/%[c]	mp/°C	$[\alpha]_D$(conc.,solv.)
Fmoc-Nva$^{\Phi}$-Sar-OBzl	97.6	38-39	-9.3 (0.3, $CHCl_3$)
Z-MeVal$^{\Phi}$-MeVal-OMe	95.4	oil	- 206 (1, MeOH)
Fmoc-Val$^{\Phi}$-MeVal-OCH_3	88.4	oil	-86.0 (0.3, $CHCl_3$)
Boc-Pro$^{\Phi}$-Pro-OBzl	90.2	oil	- 109.4 (1, $CHCl_3$)
Z-Aib$^{\Phi}$-Aib-OCH_3	95.8	108-109	—
Fmoc-MeLeu$^{\Phi}$-MeVal-OBzl	91.4	oil	-122 (0.2, $CHCl_3$)
Fmoc-MeLeu$^{\Phi}$-MeLeu-MeVal-OBzl	48.1	oil	-114.8 (1, MeOH)
Fmoc-D-Ala$^{\Phi}$-MeLeu-MeLeu-MeVal-OBzl	94.3	36-37	-145.4 (0.5, $CHCl_3$)
Boc-Val-Val-MeVal$^{\Phi}$-Pro-Pro-OBzl[d]	87.5	89-90	-181 (0.3, $CHCl_3$)

[a]The reactions were carried out as for PyBroP. In a typical experimental procedure, DIEA (3.2 equiv.) was added to a cold mixture (-10°C) of *N*-protected amino acid (1 equiv.), amino acid ester hydrochloride (1.1 equiv.), and BEP (1.1equiv.) in CH_2Cl_2 (2-4mL/mmol), stirred for 1 minute cold and for an hour at room temperature; the reaction time should be properly prolonged for the coupling between *N*-methyl amino acids. [b]The CO-NH bond formed in the peptide is indicated by Φ, all products were confirmed by ^{1}H NMR, EIMS and other characterizations. [c]Isolated yields based on *N*-protected amino acid. [d]Further confirmed by COSY and ESI-MS.

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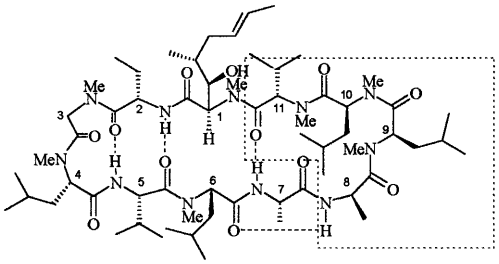
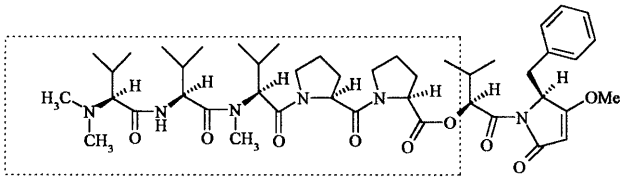
intentively protected as benzyl ester to challenge the spontaneous diketopiperazine formation. A 48% yield was still obtained during the synthesis of Fmoc-MeLeu-MeLeu-MeVal-OBzl by sequential deprotection and coupling of Fmoc-MeLeu-MeVal-OBzl with Fmoc-MeLeu-OH. According to the report by Wenger during the synthesis of cyclosporine A using modified mixed pivalic anhydride method, the same desired tripeptide was not obtained at all due to the spontaneous formation of diketopiperazine.[15] To further examine the performance of reagent BEP in SPPS, The hindered 8-11tetrapeptide of CsA and the linear undecapeptide of CsO were also synthesized in solid phase using BEP from Fmoc-MeVal-Wang resin. The purities of the obtained crude peptides were 95% and ~5%[16] respectively.



Cyclosporin A



Dolastatin 15

We also synthesized the hindered pentapeptide moiety of dolastatin 15 which was a pseudopeptide bearing promising antineoplastic activity. The Boc group was used for the N_{α}-protection, thus a 57.7% overall yield of the protected pentapeptide was obtained via seven coupling steps. No or little *N*-carboxyanhydride formed during peptide coupling using BEP, although Boc-protected amino acids were more readily converted into NCA than Cbz- or Fmoc-protected derivatives and resulted in relatively low yield using halogenerated reagents.[13d]

In general, the pyridinium type coupling reagent BEP was shown to be a very efficient coupling reagent for the synthesis of the hindered peptide containing *N*-methyl amino acid residues with fast reaction speed, low racemization and good yields.

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14 Coupling reagent BEP was prepared according to literature[9a] in 95.2% yield. mp 103-104 °C. ^{1}H NMR (300 MHz, d_6-acetone) δ 1.69 (t, J = 7.3 Hz, 3H), 5.01 (q, J = 7.3 Hz, 2H), 8.24 (m, 1H), 8.53-8.57 (m, 2H), 9.32 (d, J = 6.9 Hz, 1H) ppm. IR (KBr): ν = 3106, 1617, 1571, 1500, 1467, 1296, 1050, 786, 718, 521 cm^{-1}. FAB-MS *m/z*: 186, 188.

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16 UV analysis of the Fmoc deprotection step indicated that the coupling yield of each step was above 90%. The low purity of the crude product was most likely that the amide bonds between *N*-methyl amino acids in long peptide fragment were prone to undergo hydrolysis under strong acidic medium.[7b]