Comparative Study of Cyanuric Fluoride and BOP-Cl as Carboxyl Activators in Peptide Coupling Reactions

Young-Ah Kim and So-Yeop Han*

Department of Chemistry and Division of Molecular Life Sciences, Ewha Womans University, Seoul 120-750, Korea

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Active ester technique of organophosphorous reagents as well as acid halide technique have been widely applied to the peptide coupling reactions with steric bulkiness in organic synthesis. Commonly used coupling reagents of these types are shown in Figure 1.[1]

Active esters of amino acids produced by reacting with organophosphorous reagents usually give high regioselectivity toward nucleophilic attack by amines of other molecules in peptide coupling reactions. Especially BOP-Cl is considered as a first-choice reagent, giving high yields with negligible levels of racemization when *N*-alkylated amino acids are reacted with carbamate-protected amino acids under carefully controlled conditions.[4] Likewise, the acid halide method is frequently recommended to achieve coupling of acid-sensitive protected amino acids to sterically hindered *N*-alkyl amino acids.[5-7] Since acid chlorides are either too sensitive to be isolated or prone to decompose upon storage, acid fluorides often receive much attention due to their rapid-acting property and shelf stability. The acid fluoride is generally more popular than the acid chloride unless extremely hindered amino acid with arenesulfonyl protecting group is employed.[8]

The development of convenient and efficient methods for the total synthesis of 14-membered cyclopeptide alkaloids and epimers has been an area of focus in our laboratory. In order to complete the synthesis, we needed to practice a peptide coupling reaction between pyrrolidine moiety as a part of macrocycle and sterically hindered dipeptide as side chain. Difficulties in the coupling reaction drew our attention in seeking the best condition to succeed the synthesis by prior investigation in depth

on its kind. Less expensive and readily available cyanuric fluoride was the choice of the organohalide reagent over TFFH to find out its compatibility with the well-known organophosphorous reagent, BOP-Cl, in our system. Herein, we wish to report our results on comparative study of BOP-Cl and cyanuric fluoride as carboxyl activators in peptide coupling reactions of several model cases.

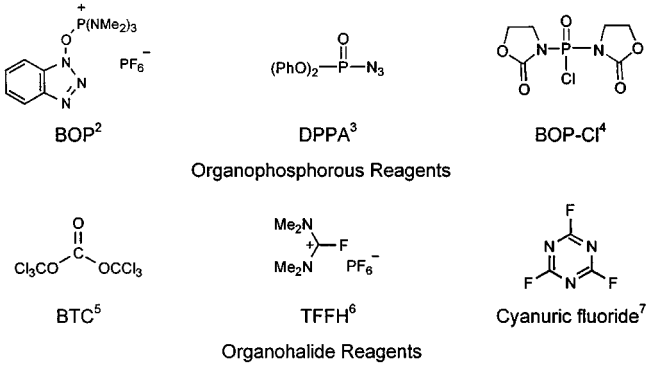
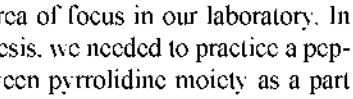


Figure 1. Representative organophosphorous and organohalide reagents.

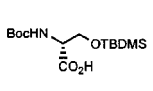
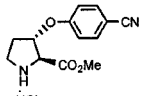
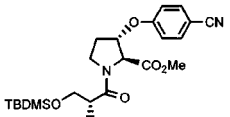
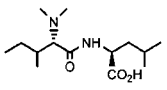
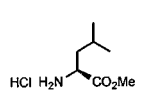
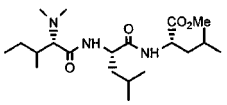
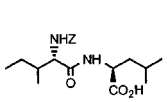
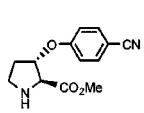
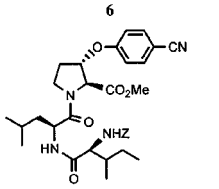
As shown in Table 1, coupling reactions were performed using BOP-Cl or cyanuric fluoride as the carboxyl activator between various amino acids. In method A, the corresponding acid fluoride was prepared in an activated form with stoichiometric amount of cyanuric fluoride in the presence of pyridine.[11] The excess coupling reagent as well as by-products were easily removed by washing the mixture with water because of weakly basic properties of the triazine ring. The resulting acid fluoride was isolated, and the crude material was subjected to further reaction with amino acid to produce the desired peptide. In method B, BOP-Cl was used as the peptide coupling reagent in the presence of DIEA. The mixed carboxylic-phosphoric anhydride intermediate was reacted with amino acid to form the corresponding peptide.

It was found that the amino acid fluoride reacted much

Table 1. Coupling Reactions of Primary or Secondary Amines with Various Carboxylic Acids



Method A: (i) Cyanuric fluoride, pyr., CH_2Cl_2, 1 h; (ii) DIEA, CH_2Cl_2. Method B: BOP-Cl, DIEA, CH_2Cl_2.

Entry	Acid (A_1)[a]	Amine (A_2)[a]	Product[b]
1			
	1	2[9]	3
2			
	4[10]	5	6
3			
	7	2	8

[a]Amino acid derivative or dipeptide fragment. [b]The yields and reaction conditions are described in Table 2.

quicker and cleaner with the nucleophilic amino components than the active ester of BOP-Cl as seen in the case of entry 1. Peptide coupling reaction between simple acid **1** and sterically hindered secondary amine **2** afforded the corresponding dipeptide **3** in 85% (method A) or 74% yield (method B), after silica-gel column chromatography. To our surprise, coupling reaction with cyanuric fluoride *via* acid fluoride was exceptionally fast at very low temperature and purification of the acid fluoride was not necessary. The optical rotation value of compound **3** was obtained within error range by both methods.

Same trend was observed when sterically hindered dipeptide acid **4** was coupled with simple amine **5** to form tripeptide **6** in entry 2. Entry 2 gave lower yields than entry 1 by both methods, indicating that the steric bulkiness of the acid component has greater impact on reactivity than that of the amine component in peptide coupling reaction. The acid **5** is one of the side chains in cyclopeptide alkaloid, which contains *N*-dimethylated amino group showing antibiotic activity against fungi and gram-positive bacteria.[12]

Formation of a peptide bond between dipeptide acid **7** and secondary amine **2** was quite challenging and required very careful control of the reaction condition (entry 3). The efficiency of cyanuric fluoride was again demonstrated by maintaining the reaction temperature below -30 °C throughout the operation[9] to minimize the side reaction. When BOP-Cl was employed as the coupling reagent, the reaction had to be carried out at or below 0 °C to avoid the formation of large quantities of by-products, causing some starting material **2** unreacted. The yield of tripeptide **8** using cyanuric fluoride was higher (59%) than BOP-Cl (39%). The formation of diastereomeric mixtures by partial racemization of the carboxyl fragment at the termini during peptide coupling reactions could easily be monitored by either HPTLC or NMR techniques. Thus HPLC analysis to evaluate the occurrence of racemization was not necessary in all experiments. Careful silica-gel flash column chromatography was sufficient enough to isolate the pure enantiomer as the major product in each experiment.

Product yields, optical rotation values and reaction conditions in peptide coupling reactions using cyanuric fluoride or BOP-Cl are summarized in Table 2. These results showed that the coupling reaction was clearly dependent on the steric factor of the two substrates. Moreover, the steric hindrance of the acid component played significant role when compared with that of the amine in chemical reactivity. Overall, use of cyanuric fluoride as in method A required comparatively shorter reaction time and led to higher yield (59-85%) than BOP-Cl as in method B (39-74%). Formation of trace amounts of diastereomers were observed on HPTLC plates in most experiments, but discarded. In fact, only in the case of entry 3, the minor diastereomer was isolated and purified by using method A. Spectral data analysis confirmed the structure of the minor isomer by comparing it with that of compound **8**. Thus, our present study share the information that while BOP-Cl is widely used in peptide coupling reactions, cyanuric fluoride gives great promise for sterically hindered peptide coupling reactions.

Table 2. Efficiency of Cyanuric Fluoride and BOP-Cl in the Formation of Peptides **3**, **6**, and **8**

Cpd	Method A[a]				Method B[b]			
	Yield[c] (%)	$[\alpha]^{25}_D$	Time[d] (h)	Temp[d] (°C)	Yield[c] (%)	$[\alpha]^{25}_D$	Time (h)	Temp (°C)
3	85	+8.0	1	-78	74	+7.8	8	0
6	71	-65.4	1	0	59	-62.3	4	rt.
8	59	-28.2	12	-78 ~ -30	39	-29.4	12	0

[a]Cyanuric fluoride as peptide coupling reagent. [b]BOP-Cl as peptide coupling reagent. [c]Isolated yields based on starting amine components. [d]Reaction time and temperature in coupling reaction of the nucleophilic amine with acid fluoride.

In conclusion, the acid fluoride obtained from cyanuric fluoride proved to be superior than active ester formed by BOP-Cl for sterically hindered peptide coupling reactions. Our experimental results can be explained in part by the following two factors. First, the steric effect in the union of the acid fluoride from cyanuric fluoride and the nucleophilic amine was minimized in order to facilitate the reaction, since the size of the fluorine atom was much smaller than the BOP group of the mixed anhydride. In addition, the strong electron withdrawing character of the fluorine atom enhanced both the reactivity of the acid fluoride toward nucleophiles as well as the ability of the leaving group.[7,13] Although peptide coupling methods using BOP-Cl and the acid fluoride from cyanuric fluoride are well-known, utilizing them in our model system was an urgent task in hoping for the completion of the synthesis of sterically hindered pyrrolidine-bearing natural product. The application of cyanuric fluoride to the peptide coupling reaction in cyclopeptide alkaloid synthesis is currently underway in our laboratory.

Experimental Section

Melting points were determined on Fisher-Johns melting point apparatus and are uncorrected. Optical rotation was determined on a Jasco DIP-140 Digital Polarimeter. BOP-Cl was purchased from Aldrich Chemical Co., cyanuric fluoride from Fluka Chemical Co., and H-Ile-Leu-OH from Bachem Chemical Co. Dichloromethane and ethylacetate were distilled from calcium hydride. Merck silica-gel 60 (230-400 mesh) was used for column chromatography. HPTLC was performed on Merck silica-gel 60 F_{254} plates. NMR spectra were recorded on a Bruker DPX 250 FT NMR using a ^1H or ^{13}C solvent peak as an internal reference. Peak assignments were based on DEPT 135, ^1H-^1H COSY and ^1H-^{13}C COSY experiments. IR spectra were recorded on a Bio-Rad FTS 165 spectrometer (KBr powder). High-resolution mass spectra (HRMS) were obtained on JMS-AX 505WA (JEOL) for chemical ionization (CI) or electron ionization (EI) and FABHRMS using the positive ion-mode with a *m*-nitrobenzyl alcohol (NBA) as the matrix.

General procedure for the preparation of dipeptide 3.

Method A: To a stirred solution of acid **1** (0.33 g, 1.0 mmol) in dry CH_2Cl_2 (5.0 mL) was added dry pyridine

(0.084 mL, 1.0 mmol) under Ar atmosphere. Cyanuric fluoride (0.10 mL, 1.0 mmol) was added dropwise to the reaction mixture at -78 °C. After 10 min, the temperature of the reaction mixture was allowed to warm to -20 °C. After stirring for 1 h, the reaction mixture containing water-soluble white precipitate was diluted with CH_2Cl_2 and washed with brine. After evaporation of the solvent, the crude acid fluoride was used further without purification. To a stirred solution of amine **2** (0.15 g, 0.52 mmol) in dry CH_2Cl_2 (2.6 mL) at -78 °C was added *N,N*-diisopropylethylamine (0.18 mL, 1.0 mmol) under Ar atmosphere. After 10 min, a solution of crude acid fluoride in dry CH_2Cl_2 (2.6 mL) was added dropwise to the reaction mixture. After stirring for 1 h, the reaction mixture was washed with brine and dried over Na_2SO_4. Solvent was removed in vacuo and the residue purified by a flash column chromatography on silica-gel (*n*-hexane : EtOAc, 3 : 1 → 5 : 2 → 2 : 1) to give dipeptide **3** (0.24 g, 85%) as a white foam. R_f 0.48 (*n*-hexane : EtOAc, 1 : 1); $[\alpha]^{25}_D$ +8.0 (*c* 1.20, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ -0.03 (6H, s, CH_3), 0.80 (9H, s, CH_3), 1.40 (9H, s, CH_3), 2.30 (2H, m, CH_2), 3.63 (1H, t, J = 8.9 Hz, CH_2), 3.77 (1H, m, CH_2), 3.79 (3H, s, OCH_3), 3.99 (2H, m, CH_2), 4.64 (2H, m, CH), 4.86 (1H, d, J = 3.4 Hz, OCH), 5.24 (1H, d, J = 8.4 Hz, NH), 6.98 (2H, d, J = 8.7 Hz, ph), 7.58 (2H, d, J = 8.6 Hz, ph); ^{13}C NMR (63 MHz, $CDCl_3$) δ -5.53, 18.19, 25.80, 28.27, 30.74, 45.39, 52.91, 53.20, 64.33, 64.71, 78.31, 79.78, 105.13, 115.88, 118.75, 134.16, 155.00, 159.70, 169.26, 170.54; HRMS (CI) $[M+H]^+$ m/z calcd 548.2794 for $C_{27}H_{42}O_7N_3Si$, found 548.2790; FTIR (KBr, cm^{-1}): 3313, 3103, 2955, 2857, 2227, 1908, 1753, 1652, 1250.

Method B: To a stirred solution of amine **2** (0.15 g, 0.51 mmol) in dry CH_2Cl_2 (2.5 mL) was added a solution of acid **1** (0.20 g, 0.62 mmol) in dry CH_2Cl_2 (2.5 mL) under Ar atmosphere, then was cooled to 0 °C. *N,N*-Diisopropylethylamine (0.18 mL, 1.0 mmol) and BOP-Cl (0.16 g, 0.62 mmol) was added to the reaction mixture. The stirring was continued for 8 h at 0 °C and the crude mixture was washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by flash column chromatography on silica-gel (*n*-hexane : EtOAc, 3 : 1 → 5 : 2 →2 : 1) to give dipeptide **3** (0.21 g, 74%) as a white foam; $[\alpha]^{25}_D$ +7.8 (*c* 2.11, $CHCl_3$). Spectral and analytical data are exactly the same as seen in method A.

Preparation of tripeptide 6.

Method A: White solid, 71% yield; R_f 0.48 (*n*-hexane : EtOAc, 1:1); mp 178-179 °C; $[\alpha]^{25}_D$ -65.4 (*c* 1.15, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.90 (18H, m, CH_3), 1.16 (H, m, CH_2), 1.59 (7H, m, CH+CH_2), 1.78 (H, m, CH), 2.19 (6H, s, NCH_3), 2.53 (1H, d, J = 5.5 Hz, CH), 3.68 (3H, s, OCH_3), 4.50 (2H, m, CH), 6.67 (1H, d, J = 8.1 Hz, NH), 6.80 (1H, d, J = 8.7 Hz, NH); ^{13}C NMR (63 MHz, $CDCl_3$) δ 12.00, 14.43, 21.65, 21.81, 22.78, 22.91, 24.67, 26.93, 29.66, 34.41, 40.41, 41.23, 43.06, 50.68, 50.92, 52.21, 74.49, 171.74, 172.01, 173.07; HRMS (EI) $[M]^+$ m/z calcd 399.3099 for $C_{21}H_{41}O_4N_3$, found 399.3105; FTIR (KBr, cm^{-1}): 3290, 3070, 2961, 1751, 1639, 1546, 1454, 1369, 1249.

Method B: 59% yield; $[\alpha]^{25}_D$ -62.3 (*c* 1.36, $CHCl_3$). Spectral and analytical data are exactly the same as seen in method A.

Preparation of tripeptide 8.

Method A: White solid, 59% yield; R_f 0.34 (*n*-hexane : EtOAc, 1 : 1); $[\alpha]^{25}_D$ -28.2 (*c* 1.40, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.84 (12H, m, CH_3), 1.02 (H, m, CH_2), 1.39 (H, m, CH_2), 1.52 (2H, m, CH_2), 1.63 (H, m, CH), 1.73 (H, m, CH), 2.31 (2H, m, CH_2), 3.76 (3H, s, OCH_3), 3.80 (1H, m, CH_2), 3.98 (1H, m, CH_2), 4.07 (1H, q, J_1 = 13.8 Hz, J_2 = 6.6 Hz, CH), 4.74 (1H, s, CH), 4.81 (H, m, CH), 4.90 (H, m, CH), 5.04 (2H, d, J = 4.6 Hz, OCH_2), 5.44 (H, d, J = 8.9 Hz, NH), 6.81 (H, d, J = 8.2 Hz, NH), 6.98 (2H, d, J = 8.4 Hz, ph), 7.28 (5H, s, ph), 7.56 (2H, d, J = 8.6 Hz, ph); ^{13}C NMR (63 MHz, $CDCl_3$) δ 11.26, 15.28, 21.76, 23.12, 24.45, 24.58, 30.62, 37.63, 41.45, 44.72, 48.57, 52.85, 59.27, 64.03, 66.78, 104.96, 115.92, 118.71, 127.81, 127.94, 128.34, 134.24, 136.15, 156.03, 159.51, 169.07, 171.01, 171.35; FABHRMS $[M+H]^+$ m/z calcd 607.3134 for $C_{33}H_{43}N_4O_7$, found 607.3121; FTIR (KBr, cm^{-1}): 3299, 2961, 2226, 1724, 1645, 1249.

Method B: 39% yield; $[\alpha]^{25}_D$ -29.4 (*c* 0.67, $CHCl_3$). Spectral and analytical data are exactly the same as seen in method A.

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References

1. Abbreviations used are: BOP, benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate; BOP-Cl, *N,N'*-bis(2-oxo-3-oxazolidinyl) phosphinic chloride; BTC, bis(trichloromethyl) carbonate; DPPA, diphenyl phosphoryl azide; TFFH, tetramethylfluoroformamidinium hexafluoro phosphate; Boc, *tert*-butyloxycarbonyl; TBDMS, *tert*-butyldimethylsilyl; Z, benzyloxycarbonyl; DIEA, *N,N*-diisopropylethylamine.
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