Tetrahedron Letters, Vol. 33, No. 5, pp. 647-650, 1992
Printed in Great Britain

0040-4039/92 $3.00 + .00
Pergamon Press plc

A NEW COUPLING REAGENT FOR PEPTIDE SYNTHESIS. BENZOTRIAZOLYLOXY-BIS(PYRROLIDINO)-CARBONIUM HEXAFLUOROPHOSPHATE (BBC)

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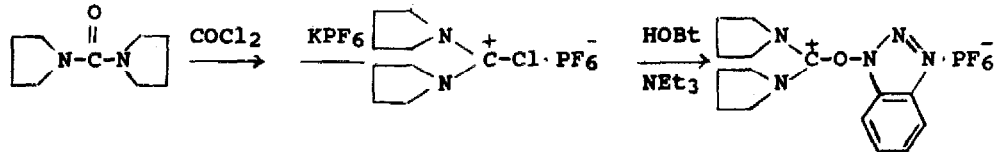
Key words
benzotriazolyloxy-bis(pyrrolidino)-carbonium hexafluorophosphate (BBC); coupling reagent; solution and solid phase peptide synthesis; Leu-enkephalin

Abstract: Benzotriazolyloxy-bis(pyrrolidino)-carbonium hexafluorophosphate (BBC) is found to be a new excellent coupling reagent devoid of cytotoxic by-product instead of HBTU and BOP. It has been applied in the solution and solid phase peptide synthesis.

Recently, many coupling reagents are reported to be used for peptide synthesis[1]. Among them, O-benzotriazolyl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) is an excellent peptide coupling reagent[2-4]. However, in the manufacture of HBTU, as well as its utilization, tetramethylurea is used or formed. It is volatile and has been reported to be cytotoxic[5]. In this paper, we report a new reagent, benzotriazolyloxy-bis(pyrrolidino)-carbonium hexafluorophosphate(BBC), to be used as a more efficient peptide coupling reagent devoid of the by-product tetramethylurea.

BBC was prepared from the non-volatile 1,1'-carbonyl dipyrrolidine. A 20% solution of phosgene in toluene (8 mL, 16 mmol) was added to a solution of 1,1'-carbonyl dipyrrolidine **1** (2 g, 12 mmol) in toluene. After approximately 0.5 h, the excess of phosgene was removed under reduced pressure. Anhydrous ether was added under vigorous stirring to give a white solid salt. The salt was dissolved in water. To this solution was added a saturated solution of KPF_6 (2.2 g, 12 mmol) under continous stirring to form white precipitate, which upon recrystallization from acetone/ether to yield 3.2 g (81%) of pure **2**: m.p.146-148°C. A solution of **2** (2.5 g, 7.5 mmol) in dichloromethene and hydroxybenzotriazole monohydrate (1.15 g, 7.5 mmol) and triethylamine (1.0 mL, 7.5 mmol) was stirred approximately for 10 h. The solution was diluted with anhydrous ether to give solid residue which was washed with water, dried and recrystallized from acetone/ether to yield **3** (BBC): 2.94 g (93%);

m.p.178-180°C; ^{1}HNMR(d_6-acetone): 8.12-7.56(4H, m), 4.60-3.55 (8H, m), 2.60-1.80(8H, m)). BBC is non hygroscopic, stable and can be stored at room temperature.



1 2 3 (BBC)

Coupling tests using BBC in solution are shown in Table 1. In the DMF solution containing 1 equiv. N-protected amino acid and 1 equiv. C-protected amino acid hydrochloride or trifluoroacetic acid (TFA) salt, 1.05 equiv. BBC and 3 equiv. diisopropylethylamine (DIEA) were added. TLC monitoring showed that it reacted immediately.

Table 1. Preparation of peptides with BBC in DMF[a]

No.	Peptide	Yield(%)[b]	M.p.°C	$[\alpha]_D$(C,solvent,°C)[c]
1	Boc-Phe-Leu-OMe	89.7	102-103	-24.3(1,MeOH,18)
2	BOC-Ile-Cys(Bzl)-OMe	85.2	115-117	-64.8(1,MeOH,18)
3	Boc-Tyr(Bzl)-Gly-OEt	94.5	126-127	+1.0(1,MeOH,20)
4	Boc-Tyr(Bzl)-Gly↓-Gly-OEt[d]	95.3	102-104	+13.4(1,MeOH,15)
5	Boc-Phe-Phe-OMe	96.0	121-123	-13.7(1,MeOH,27)
6	Boc-Tyr(Bzl)-Gly-Gly↓-Phe-Leu-OMe[d]	90.9	146-148	-7.7(1,MeOH,18)
7	Boc-Leu-Trp-OMe	92.8	92-94	-13.4(1,MeOH,27)
8	Boc-Ile-Met-OMe	87.5	118-120	-48.0(1,MeOH,27)
9	Boc-Phe-Met-OMe	90.2	91-92	-20.9(1,MeOH,27)

a. The reactions were performed with equimolar amounts of a carboxyl component, an amino component hydrochloride (TFA salt in No. 6), 1.05 equiv. BBC and 3 equiv. DIEA in DMF at room temperature for 15 min. b. The yields refer to isolated products. c. Melting points and $[\alpha]_D$ values are in accord with the reported values and all the products are confirmed by elemental analysis. d. The arrow indicates a coupling site.

Using HPLC method[6] (the coupling of Z-Gly-Phe-OH and Val-OMe·HCl) and Young test[7] (the coupling of Bz-Leu-OH and Gly-OEt·HCl), the extent of the racemization with the reagent BBC was examined. Comparing with the other same-type reagents and DCCI, the racemization with BBC was observed to be the lowest as shown in Table 2.

Table 2. Comparison of racemizations of different methods

Reagent	HPLC method	Young test	
	DL%	$[\alpha]_D$	DL%
DCCI	18.8	-9.0	73.6
BOP	9.6	-20.5	39.8
PyBOP[a]	6.6	-24	30
HBTU	9.0	-25.4	25.4
BBC	6.4	-27.6	18.8

a. The reactions with all reagents were carried out in the same conditions except PyBOP where the data were from Lit.,[1d].

It has been reported that the coupling rate of HBTU is the greatest of many reagents including BOP[4]. In this paper, we compared the new reagent BBC with HBTU in the same conditions and found that the coupling rate of BBC was even greater than that of HBTU (Table 3). The coupling efficiency was determined by the quantitative ninhydrin method[8].

Table 3. Comparison of coupling rate between BBC and HBTU[a]

Time (min.)		1	2	5	10	30
Uncoupled amine	HBTU	4.3%	0.84%	0.63%	0.48%	0.42%
	BBC	0.32%	0.32%	0.26%	0.25%	0.18%

a. Reaction condition: 0.06 mmol Gly-OCH_2-resin, 3 equiv. Boc-Ile and 3 equiv. BBC, 6 equiv. DIEA in 5 mL DMF.

In order to evaluate the application of the reagent BBC in the peptide chemistry, Leu-enkephalin was synthesized both in the solution and in the solid phase peptide synthesis. The HPLC profiles showed that the products synthesized by the two methods were identical with the authentic sample.

Thus, it has been proved that BBC is superior to HBTU and BOP and it seems to be a more efficient reagent for peptide synthesis.

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(Received in Japan 15 October 1991)