Application of DEPBT on the Synthesis of the Protected Dipeptides Containing Histidine with Unprotected Imidazole Group by Solution Method†

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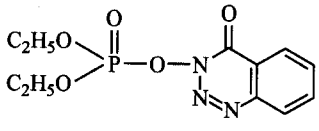
3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) was an organophosphorus coupling reagent developed by our group. It was an effective coupling reagent for the synthesis of protected peptides containing Tyr, Ser and Thr with unprotected hydroxy group on their side chain. The further study of the synthesis of a series of protected dipeptides containing histidine with unprotected imidazole group using DEPBT is reported. During the synthetic procedure, the imidazole group of histidine did not need to be protected. When the carboxyl components were *N*-protected aromatic amino acids or basic amino acids, the yields were relatively high (63%—81%). However, when the carboxyl components were *N*-protected acidic amino acids, the yields were relatively low (47%—48%). The results expanded the application of DEPBT on the synthesis of bioactive peptides containing histidine.

Keywords DEPBT, coupling reagent, histidine, peptide

Introduction

3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) is an organophosphorus coupling reagent developed by our group for the synthesis of peptides.[1-3]



DEPBT

It is a colorless stable crystal and can be kept at room temperature for several months without any decomposition. Furthermore, it is easy to be prepared and can be soluble in common organic solvents, such as THF, CH_2Cl_2, *etc*. DEPBT has been successfully used for the synthesis of linear peptide by both solution method and solid-phase method. It is also an effective coupling reagent for the synthesis of cyclic peptide in one-pot procedure.[4,5] During the coupling procedure, side reaction and racemization are minimal. The racemization caused by DEPBT in DMF was less than 1% detected by Young test. Under the same condition, 18.4% of *D*-isomer was obtained when BOP was used.[2] *D*-Isomer caused by DEPBT was 0%—5% in different conditions examined by chiral HPLC. However, when BOP, HBTU, HATU and Py-Brop were used, more than 15% of *D*-isomer was formed.[6] Moreover, It is not necessary to protect the hydroxy group of the amino component such as Ser, Thr and Tyr. Based on these advantages, we reported the use of DEPBT on the synthesis of a series of peptide alcohols and an *N*-glycopeptide derivative from *N*-protected amino acids as carboxyl components and different amino components with one or more unprotected hydroxy groups.[7,8] DEPBT was also used for the synthesis of complex natural products. It was utilized for amide bond formation in the total synthesis of (−)-tamandarin B[9] and the glycopeptide teicoplanin aglycon[10] with high conversions, and most importantly, with no detectable racemization. While other reagents provided occasionally low conversions or significant racemization.

Histidine exists in active center of many enzymes and proteins, and many bioactive peptides contain histidine. It acts as an important role in the acid-base catalysis of enzymes. So the synthesis of bioactive peptides containing histidine is very important for the investigation on the structure-bioactivity relationship of some enzymes and bioactive peptides. However, the imidazole group of histidine is a weak base group, thus it is easy to generate side reactions during coupling procedure, such as alkylation, acylation on the imidazole group. Furthermore, it is very easy to racemize. Therefore, in the synthesis of peptides, it is usually necessary to protect the imidazole group of histidine when general coupling reagents are used.[11,12]

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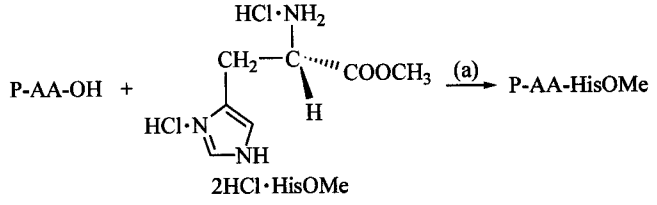
In this paper, we report the synthesis of a series of protected dipeptides from different *N*-protected amino acids as carboxyl component and HisOMe·2HCl with unprotected imidazole group as amino component (Scheme 1). During the synthetic procedure, the imidazole group of histidine did not need to be protected. The results indicate that the application of DEPBT can be widely used for the synthesis of bioactive peptides containing histidine with unprotected imidazole group.

Scheme 1 Synthesis of protected dipeptides containing histidine with unprotected imidazole group using DEPBT



(a) DEPBT, Et_3N, DMF
P = Z, Boc; AA = Amino acid residue

Experimental

The melting points were determined with a Yanaco micromelting point apparatus and uncorrected. Mass spectra were recorded on a ZAB-HS spectrometer (Micromass, Manchester, UK). And the data of HRMS were recorded by APEX II FT-ICR-MS spectrometer (Bruker Daltonics Inc. Billerica, MA, USA) using L-SIMS ionization. ^1H NMR spectra were recorded on a Bruker ARX-400 spectrometer. Elemental analysis was performed by an Elementar Vario EL elemental analyzer (Germany). Optical rotations were measured by a Perkin Elmer 341LC polarimeter. Abbreviations: Standard abbreviations for amino acids and peptide derivatives are based on the suggestions of the IUPAC-IUB Commission on Biochemical Nomenclature *Eur. J. Biochem.* **1984**, *138*, 9—37. Other abbreviations: BOP, benzotriazol-1-yloxytris(dimethylamino)Phosphonium hexafluorophosphate; HBTU, *O*-Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; Py-Brop, bromotris-(1-pyrrolidino), phosphonium hexafluorophosphate; Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; DMF, *N*,*N*-dimethylformamide.

General experimental procedure

DEPBT (0.11 mmol/mL) was added to a suspension of the carboxyl component (0.1 mmol/mL), HisOMe·2HCl (0.1 mmol/mL) and Et_3N (0.21 mmol/mL) in DMF. The pH of solution was adjusted to 8—9 with Et_3N. The mixture was stirred at room temperature for about 12 h and monitored by TLC. Then the mixture was evaporated *in vacuo* and the residue was dissolved in 100 mL of ethyl acetate. The solution was successively washed with saturated NaCl, 5% Na_2CO_3, and saturated NaCl solution. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The crude product was recyrstallized from suitable mixed solvents or purified by silica gel chromatography to afford pure product.

The following compounds were prepared using above procedure, and the yields and MS of all compounds are listed in Table 1.

Boc-Tyr(Bzl)-HisOMe White solid, m.p. 122—123 ℃, $[\alpha]_D^{20}$ −1.5 (*c* 1.0, EtOH). Anal. calcd for $C_{28}H_{34}N_4O_6$: C 64.35, H 6.56, N 10.72; found C 64.53, H 6.74, N 10.49.

Boc-Ser(Bzl)-HisOMe White solid, m.p. 70—72 ℃, $[\alpha]_D^{20}$ + 59.6 (*c* 1.0, $CHCl_3$). Anal. calcd for $C_{22}H_{30}N_4O_6$: C 59.18, H 6.77, N, 12.55; found C 58.96, H 6.77, N 12.37.

Boc-Glu(OBzl)-HisOMe Colorless oil, $[\alpha]_D^{20}$ −9.2 (*c* 0.9, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.43 (s, 9H, 3 × CH_3), 1.90—2.04 (m, 1H, CH_2), 2.04—2.19 (m, 1H, CH_2), 2.40—2.60 (m, 2H, CH_2), 3.11—3.21 (m, 2H, CH_2), 3.71 (s, 3H, OCH_3), 4.15 (dd, *J* = 7.4, 13.6 Hz, 1H, CH), 4.79 (dd, *J* = 4.8, 12.2 Hz, 1H, CH), 5.12 (s, 2H, CH_2Ph), 6.82 (s, 1H, CH), 7.23—7.42 (m, 5H, ArH), 7.63 (s, 1H, CH). HRMS calcd for $C_{24}H_{32}N_4O_7$ 489.2344 $(M+H)^+$, found 489.2343.

Boc-Asp(OBzl)-HisOMe White solid, m.p. 66—68 ℃, $[\alpha]_D^{20}$ −9.4 (*c* 1.0, MeOH). Anal. calcd for $C_{23}H_{30}N_4O_7$: C 58.22, H 6.37, N 11.81; found C 57.67, H 6.40, N 11.46.

Boc-Phe-HisOMe White solid, m.p. 136—137 ℃, $[\alpha]_D^{20}$ −7.0 (*c* 1.1, MeOH). Anal. calcd for $C_{21}H_{28}N_4O_5$: C 60.56, H 6.78, N 13.45; found C 60.60, H 6.86, N 13.52.

Boc-Pro-HisOMe Colorless oil, $[\alpha]_D^{20}$ −46.9 (*c* 0.7, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.47 (s, 9H, 3 × CH_3), 1.74—1.94 (m, 1H, CH_2), 1.98—2.06 (m, 1H, CH_2), 2.06—2.18 (m, 1H, CH_2), 3.16 (d, *J* = 15.0 Hz, 1H, CH_2), 3.33 (d, *J* = 12.0 Hz, 1H, CH_2), 3.40—3.49 (m, 1H, CH_2), 3.49—3.60 (m, 1H, CH_2), 3.75 (s, 3H, OCH_3), 4.07—4.20 (m, 1H, CH), 4.70—4.85 (m, 1H, CH), 6.77 (s, 1H, CH), 7.59 (s, 1H, CH). HRMS calcd for $C_{17}H_{26}N_4O_5$ 367.1976 $(M+H)^+$, found 367.1972.

Boc-Trp-HisOMe White solid, m.p. 118—120.5 ℃, $[\alpha]_D^{20}$ −16.0 (*c* 0.7, MeOH) [Lit.[13] m.p. 113.5—115 ℃, $[\alpha]_D^{22}$ −17.3 (*c* 1.74, MeOH)].

Boc-Arg(Tos)-HisOMe Colorless oil, $[\alpha]_D^{20}$ −2.9 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.41 (s, 9H, 3 × CH_3), 1.45—1.78 (m, 4H, 2 × CH_2), 2.94—3.34 (m, 4H, 2 × CH_2), 3.68 (s, 3H, OCH_3), 4.14 (brs, 1H, CH), 4.77 (brs, 1H, CH), 6.30—6.66 (m, 3H, ArH, CH_2Ph), 6.88 (s, 1H, CH), 7.23 (d, *J* = 8.1 Hz, 2H, ArH), 7.68 (s, 1H, CH), 7.75 (d, *J* = 7.9 Hz, 2H, ArH). HRMS calcd for

Table 1 Yields of protected dipeptides containing histidine with unprotected imidazole group synthesized by DEPBT

No.	Carboxyl component	Product	FAB-MS m/z $(M+H)^+$	Yield (%)
1	Boc-Tyr(Bzl)-OH	Boc-Tyr(Bzl)-HisOMe	523	81.0
2	Boc-Ser(Bzl)-OH	Boc-Ser(Bzl)-HisOMe	447	54.6
3	Boc-Glu(OBzl)-OH	Boc-Glu(OBzl)-HisOMe	489	48.5
4	Boc-Asp(OBzl)-OH	Boc-Asp(OBzl)-HisOMe	475	47.3
5	Boc-Phe-OH	Boc-Phe-HisOMe	417	63.7
6	Boc-Pro-OH	Boc-Pro-HisOMe	367	56.1
7	Boc-Trp-OH	Boc-Trp-HisOMe	456	70.7
8	Boc-Arg(Tos)-OH	Boc-Arg(Tos)-HisOMe	580	64.7
9	Boc-Lys(2-Cl-Z)-OH	Boc-Lys(2-Cl-Z)-HisOMe	566	75.0
10	Z-Phe-OH	Z-Phe-HisOMe	451	70.0

$C_{25}H_{37}N_7O_7S$ 580.2548 $(M+H)^+$, found 580.2540.

Boc-Lys(2-Cl-Z)-HisOMe White solid, m.p. 100—105 ℃, $[\alpha]_D^{20}$ −9.1 (*c* 0.9, MeOH). Anal. calcd for $C_{26}H_{36}ClN_5O_7$: C 55.17, H 6.41, N 12.37; found C 55.14, H 6.42, N 11.88.

Z-Phe-HisOMe White solid, m.p. 124—126 ℃, $[\alpha]_D^{20}$ −18.6 (*c* 1, DMF) [Lit.[14] m.p. 121—124 ℃, $[\alpha]_D^{20}$ −19.0 (DMF)].

Results and discussion

A series of protected dipeptides were synthesized by DEPBT from different *N*-protected amino acids as carboxyl components and HisOMe·2HCl with unprotected imidazole group as amino component (Scheme 1). The yields of these protected dipeptides are shown in Table 1. The results showed that DEPBT was an efficient coupling reagent for amide formation from amino component with unprotected imidazole group. The expected products were obtained in moderate yields. In contrast with the synthetic procedure for synthesis of peptides containing histidine by using general coupling reagents, the protecting and deprotecting reactions for imidazole group of histidine could be omitted by using DEPBT. This result expanded the application of DEPBT on the synthesis of bioactive peptides containing histidine.

In conclusion, DEPBT was an excellent coupling reagent for the synthesis of peptides containing histidine. When DEPBT was used as coupling reagent, carboxyl components selectively reacted with NH_2 of amino component histidine in presence of unprotected imidazole group.

As shown in Table 1, when the carboxyl components were *N*-protected aromatic amino acids (such as Tyr, Phe and Trp) or *N*-protected basic amino acids (such as Arg and Lys), the yields were relatively high (63%—81%). Among them, the synthetic yield of Boc-Tyr(Bzl)-HisOMe was the highest (81%). However, when the carboxyl components were *N*-protected acidic amino acids (such as Glu and Asp), the yields were relatively low (47%—48%).

When histidine methyl ester was amino component and Boc-Ser-OH, Boc-Tyr-OH and Z-Tyr-OH with unprotected hydroxy group were used as carboxyl components, the yields of the expected products were too low to obtain the pure products. The existence of the expected products could only be detected by FAB-MS. This result was probably caused by the catalytic effect of unprotected imidazole group of histidine in the *O*-acylation of amino acid with a hydroxy group in their side chain.[12] Therefore, the hydroxy groups of carboxyl components usually need to be protected when histidine derivatives with unprotected imidazole group were used as amino components.

DEPBT had been widely used in the synthesis of peptides with different *N*-protected amino acids containing neutral fatty amino acids as the carboxyl components. The coupling yields were generally between 65% to 95%. While *N*-protected neutral fatty amino acids were not suitable to react with histidine ester with unprotected imidazole group as amino component when using DEPBT in our experiment. The further study of the effect of carboxyl components on the synthesis of peptides containing histidine with unprotected imidazole group by DEPBT is underway.

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