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Facile Synthesis of *N-tert*-Butyloxycarbonyl-*O*-benzyl-L-threonine

Kazuo UEDA*

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Abstract

A facile procedure for the synthesis of *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine derived from L-threonine is described. *N-tert*-Butyloxycarbonyl-*O*-benzyl-L-threonine is obtained in an excellent yield by the reaction of *N-tert*-butyloxycarbonyl-L-threonine with sodium hydride and benzyl bromide and subsequent saponification with sodium hydroxide. The procedure is simple and the reaction proceeds without observable racemization.

In peptide synthesis, especially using the solid-phase method, *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine has proved to be a useful intermediate to incorporate threonine residue into a synthetic peptide.[1] The synthesis of *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine is difficult and cannot be accomplished with yields comparable to those obtained with serine. Mizoguchi *et al.*[2] prepared *O*-benzyl-L-threonine benzyl ester by long heating of L-threonine with benzyl alcohol and *p*-toluenesulfonic acid. Following saponification of the benzyl ester and conversion to the *N-tert*-butyloxycarbonyl derivative, the overall yield obtained was only about 10%. The method of Sugano and Miyoshi[3] may be slightly better, offering a 14% yield of *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine from *N-tert*-butyloxycarbonyl-L-threonine by treatment of the latter compound with 2 molar equivalent of sodium hydride and 1 molar equivalent of benzyl bromide.

This paper is concerned with the development of a more improved synthetic method of *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine starting from L-threonine.

A route of synthesis is shown in the Scheme. *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine was obtained from the readily available *N-tert*-butyl-

*Department of Chemistry, Faculty of Liberal Arts, Nagasaki University

$$\text{H-L-Thr-OH} \xrightarrow[\text{TEA}]{\text{Boc-ON}} \text{Boc-L-Thr-OH}$$

$$\xrightarrow[\text{iii) } OH^-,\ \text{iv) } H^+]{\text{i) NaH, ii) Benzyl bromide}} \text{Boc-L-Thr(Bzl)-OH}$$

Scheme. Synthetic route of *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine [Boc-L-Thr(Bzl)-OH].[4]

oxycarbonyl-L-threonine by treatment of the latter first with 2 molar equivalent of sodium hydride and then with 2 molar equivalent of benzyl bromide, and following saponification of the benzyl ester in the reaction mixture with 1 molar equivalent of sodium hydroxide. By the use of column chromatography for purification, *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine was obtained in good yield of 52%. The method described here is suitable for large-scale preparation because of its efficiency, procedural simplicity, and mildness of reaction conditions, in which optical purity is maintained.

When the same procedure was applied to the preparation of *N-tert*-butyloxycarbonyl-*O*-benzyl-L-serine, the yield of the compound from *N-tert*-butyloxycarbonyl-L-serine was 51% as its cyclohexylammonium salt.

Experimental Section[5]

N-tert-*Butyloxycarbonyl-L-threonine Dicyclohexylammonium Salt.* L-Threonine was converted to the desired product by the method of Itoh *et al.*[6] (reaction with 2-(*tert*-butyloxycarbonyloxyimino)-2-phenyl-acetonitrile in the presence of triethylamine) in 99% yield. mp 151.0–152.0°C. Lit.,[6] mp 151–153°C.

N-tert-*Butyloxycarbonyl*-O-*benzyl-L-threonine.* Crystalline *N-tert*-butyloxycarbonyl-L-threonine dicyclohexylammonium salt was converted to an oily *N-tert*-butyloxycarbonyl-L-threonine with 1N HCl. To a solution of *N-tert*-butyloxycarbonyl-L-threonine (2.19 g, 10.0 mmol) in dry dimethylformamide (50 ml) was added sodium hydride (50%) (1.06 g, 22.0 mmol) at 0°C. After the evolution of hydrogen gas ceased, the freshly distilled benzyl bromide (2.61 ml, 22.0 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 5 h to give a clear solution. The solvent was then removed under reduced pressure at 30°C. The resulting residue was dissolved in methanol (20 ml) and treated with 1N sodium hydroxide (12.0 ml, 12.0 mmol). After the solution had stood for 2 h at room

temperature, it was evaporated and the residue was dissolved in water (50 ml). The aqueous solution was extracted with diethyl ether (two 20-ml portions). The aqueous phase was chilled, acidified to pH 3 with solid citric acid, and extracted with ethyl acetate (two 50-ml portions). The combined organic layers were washed successively with water and saturated sodium chloride solution and dried over anhydrous magnesium sulfate. The ethyl acetate was removed under reduced pressure to give a pale yellow oil. The oily product was dissolved in chloroform (7 ml), placed on a 2 × 15 cm column of silica gel (Wakogel C-200), and eluted with chloroform (300 ml). The chloroform was evaporated in vacuo to give a desired *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine as a colorless oil which crystallized from petroleum ether (bp 30–60°C). Recrystallization from ethyl acetate–petroleum ether yielded the title compound (1.61 g, 52%) : mp 115.0–115.5°C; $[\alpha]_D^{17}$ + 15.0° (*c* 4, methanol). Lit.,[2] mp 115–116°C, $[\alpha]_D^{22}$ + 15.8° (*c* 1.1, methanol). The product was homogeneous by thin layer chromatography (silica gel) and showed R_f 0.92 in 1-butanol–acetic acid–water (4 : 1 : 2, v/v) ; R_f 0.62 in chloroform–methanol (5 : 1, v/v) ; and R_f 0.80 in 1-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 12, v/v).

Anal. Calcd for $C_{16}H_{23}NO_5$: C, 62.12 ; H, 7.49 ; N, 4.53%. Found : C, 62.02 ; H, 7.52 ; N, 4.55%.

Demonstration of Optical Purity. An aliquot of *N-tert*-butyloxycarbonyl-*O*-benzyl-L-threonine prepared by the above procedure was dissolved in 3 N HCl/dioxane. After 3 h at room temperature, the solvent was evaporated under reduced pressure at 30°C to yield the solid which was dried in vacuo over potassium hydroxide. The recovered material showed the same optical rotations as a sample of *O*-benzyl-L-threonine[2] similarly treated, $[\alpha]_D^{21}$ − 28.0° (*c* 4, acetic acid). Paper chromatography of the recovered material in the system 1-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 12, v/v) revealed only *O*-benzyl-L-threonine (R_f 0.64).

References and Notes

1) R. B. Merrifield, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 32, 221 (1969).
2) T. Mizoguchi, G. Levin, D. W. Woolley, and J. M. Stewart, *J. Org. Chem.*, 33, 903 (1968).
3) H. Sugano and M. Miyoshi, *J. Org. Chem.*, 41, 2352 (1976).
4) Abbreviations : Boc, *tert*-butyloxycarbonyl ; Boc-ON, 2-(*tert*-butyloxycarbonyloxyimino)-2-phenyl-acetonitrile ; Bzl, benzyl ether ; TEA, triethylamine.

5) All melting points were determined in capillaries on a MP-21 Yamato melting point apparatus and are uncorrected. Optical rotations were measured on an Atago polarimeter polax using 1 dm tube at room temperature. Thin layer chromatography was performed on silica gel 60 F_{254} pre-coated plates (Merck). Paper chromatography was carried out on Toyo Roshi No. 50 paper.

6) M. ITOH, D. HAGIWARA, and T. KAMIYA, *Bull. Chem. Soc. Jpn.*, **50**, 718 (1977).