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PROTECTION OF THE TRYPTOPHAN INDOLE RING IN PEPTIDE SYNTHESIS. USE OF A NEW DERIVATIVE, N^{in}-2,2,2-TRICHLOROETHOXYCARBONYLTRYPTOPHAN

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The 2,2,2-trichloroethoxycarbonyl (Troc) group attached at the N^{in} function of tryptophan by acylation with Troc-Cl in the presence of a catalytic amount of tetra-n-butylammonium hydrogensulfate can be quantitatively removed either by cadmium dust in acetic acid or under basic conditions, e.g., hydrazine hydrate and NaOH, but is resistant to strong acidic conditions.

The indole moiety of tryptophan is susceptible to oxidation and to attack by cations during the acid treatment for removal of N^{α} protecting groups in peptide synthesis.[1] To avoid these side reactions, protection of tryptophan by attachment of an electron withdrawing group at the indole nitrogen atom is desirable. However, only a very few investigations have been concerned with N^{in} protecting groups because of the difficulty to introduce the protecting groups into the indole nitrogen atom.[2-4]

We now report that the 2,2,2-trichloroethoxycarbonyl (Troc) group[5] attached at the N^{in} function of tryptophan is useful as a protecting group in peptide synthesis. The key feature is the introduction of Troc group into the indole nitrogen atom by the acylation reaction using a catalytic amount of tetra-n-butyl ammonium hydrogensulfate (TBAHS).[6] Very recently, 4-methoxy-2,3,6-trimethylbenzenesulfonyl group[4] has been reported for the same purpose.

The Troc group was introduced by acylation of Boc-Trp-OBzl[7] (1 equiv.) using Troc-Cl[8] (1.5 equiv.)(Aldrich Chemical Co.) in methylene chloride in the presence of pulverized NaOH (2.5 equiv.) and TBAHS (0.01 equiv.) at room temperature for 2 h (Scheme 1). Boc-Trp(Troc)-OBzl (m.p. 122-125°C) was readily obtained in 92% yield as a white crystalline compound (crystallized from methanol) without any troublesome purification procedures. Boc-Trp(Troc)-OBzl was quantitatively debenzylated into Boc-Trp(Troc) (mp 186-188°C, isolated yield 94%)

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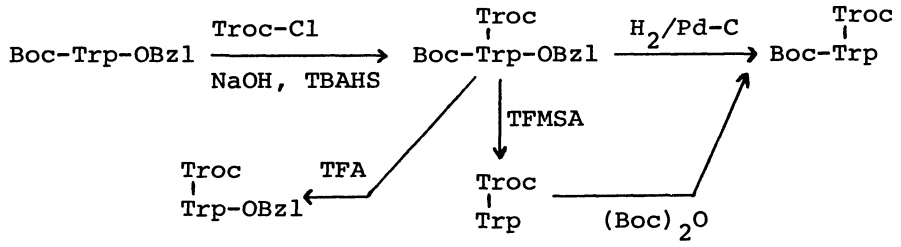
Scheme 1. Boc = t-butoxycarbonyl, Trp = L-tryptophan, Bzl = benzyl, Troc = 2,2,2-trichloroethoxycarbonyl, TBAHS = tetra-n-butylammonium hydrogensulfate, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, $(Boc)_2O$ = di-t-butyl dicarbonate.



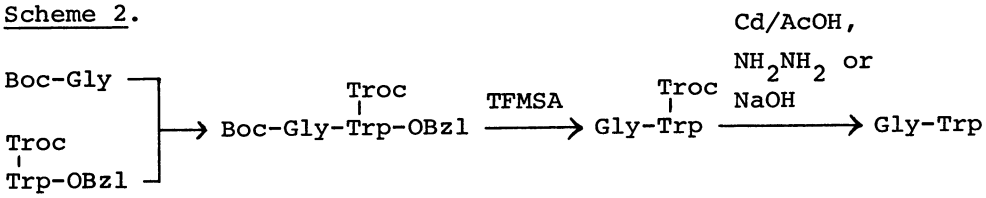
by catalytic hydrogenation on 5% Pd-C at room temperature and atmospheric pressure for 1 h in tetrahydrofuran. During this catalytic hydrogenolysis, N^{in}-Troc group was stable and no hydrogenation of the indole into a 2,3-dihydroindole side chain was observed,[9] but in the case of longer exposure (about 3 hr) to hydrogen gas in the presence of 5% Pd-C, very small amounts of impurities appeared in t.l.c.

Trp(Troc) (mp 222-225°C) was obtained as a white crystalline compound by the deprotection reaction of Boc-Trp(Troc)-OBzl with trifluoromethanesulfonic acid (TFMSA) - trifluoroacetic acid (TFA) - o-cresol at room temperature for 4 h. The N^{in}-Troc group was stable to such strong acidic conditions as TFMSA, and no side reactions on the indole ring were detected. Boc-Trp(Troc) described above was also obtained in another method from Trp(Troc) (1 equiv.) by t-butoxycarbonylation using $(Boc)_2O$[10] (1 equiv.) and triethylamine (1 equiv.) in dimethyl sulfoxide.

The N^{in}-Troc group could be completely removed either under basic conditions, i.e., 0.01 M NaOH (1.1 equiv.) in methanol for 2 h and hydrazine hydrate (20 equiv.) in methanol - water for 4 h at room temperature, or by cadmium dust in acetic acid - dimethylformamide (DMF) for 3 h at room temperature.[11] However, the deprotection of Trp(Troc) was incomplete in Zn - acetic acid - DMF after 20 h at room temperature, while the Troc group attached at the α-amino[5,8,12] and hydrazide[13] function was easily deprotected in Zn - acetic acid.

As a model compound, Boc-Gly-Trp(Troc)-OBzl was prepared with dicyclohexylcarbodiimide by the condensation of Boc-Gly and Trp(Troc)-OBzl·TFA [converted

Scheme 2.



from Boc-Trp(Troc)-OBzl by TFA - anisole] in the presence of triethylamine (Scheme 2). This protected peptide was converted into Gly-Trp(Troc) by acidolysis using TFMSA - TFA - dimethyl sulfide, and removal of the N^{in}-Troc group was demonstrated by three methods: hydrazine hydrate, NaOH, and cadmium dust. Boc-Trp(Troc)-Gly-OEt was further synthesized with norborn-5-ene-2,3-dicarboximido diphenyl phosphate (NDPP)[14] by the condensation of Boc-Trp(Troc) and Gly-OEt·HCl in the presence of triethylamine.

Thus, by the use of the acid-stable N^{in}-Troc group as a protecting group, the naked indole moiety has not been exposed to acidic conditions during the syntheses of peptides at all, and side reactions on the indole moiety of tryptophan have been prevented because of the reduction of electron density on the indole ring by using the electron-withdrawing Troc group.

Scheme 3. Z = benzyloxycarbonyl.

It is noteworthy that the introduction of benzyloxycarbonyl (Z) group into the indole nitrogen atom of tryptophan proceeded also conveniently using a catalytic amount of TBAHS. Boc-Trp(Z)-OMe (mp 118-120°C) was readily obtained in 84% yield as a crystalline compound from Boc-Trp-OMe (1 equiv.) by benzyloxycarbonylation using Z-Cl (1.5 equiv.), pulverized NaOH (2.5 equiv.) and TBAHS (0.01 equiv.) in methylene chloride at room temperature for 2 (Scheme 3). On the other hand, in the case of benzyloxycarbonylation of the indole ring using crown ethers,[3] use of Z-Cl as an acylating reagent resulted in poor yields, and that of benzyloxycarbonyl p-nitrophenyl carbonate required longer reaction time and troublesome purification procedures. Boc-Trp(Z)-OMe was converted into Trp(Z)-OMe·TFA by TFA - anisole at 0°C for 1 h, and the debenyloxycarbonylation of this compound was incomplete even after 24 h at room

temperature in TFA - thioanisole which could deprotect N^{α}- and N^{ε}-Z group.[15] This result indicates that the N^{in}-Z group is more stable to acidic conditions than the N^{α}- or N^{ε}-Z group.

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