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A NEW METHOD FOR THE SOLID PHASE SYNTHESIS OF TRYPTOPHAN CONTAINING PEPTIDES*

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Solid phase synthesis of tryptophan containing peptides with use of diphenylphosphinothioyl(Ppt)-amino acids is described. Ppt group could be removed from tryptophyl peptides without coloration by 2*N* HCl in methylene chloride containing 1*M* triphenylphosphine.

Synthesis of tryptophan containing peptides is not easy because of the undesired side reactions.[1] To avoid them deprotection is usually performed in the presence of scavengers. Protection by formylation of indole nitrogen has also been reported.[2] Better solution of this problem should exist in adopting a protecting group which is removable under the reductive conditions and produces no reactive species by cleavage. In this communication application of diphenylphosphinothioyl(Ppt) group[3] for solid phase synthesis of tryptophyl peptides is described.

Ppt group can be removed by the same reagents used for the removal of *t*-butyloxycarbonyl(Boc) group, while some preference of hydrogen chloride in acetic acid or dioxane to trifluoroacetic acid was observed. Methylene chloride is the most favorable solvent for solid phase synthesis, but HCl is almost insoluble in this solvent. Then, use of HCl as the salt of triphenylphosphine(TPP) was considered. TPP is reported to give dihydrochloride (I).[4] I is hygroscopic a little and decomposed by water to TPP. I was found to be soluble in methylene chloride, so the solution of I was prepared directry by passing dry HCl to the solution of TPP in methylene chloride. Deprotection of Ppt-L-Trp-OMe was tried using 1*N* HCl in methylene chloride containing 0.5*M* TPP. Ppt group was cleaved completely within 30 min at 30°C. As expected deprotection product, Ppt-Cl, did not affect the indole moiety of tryptophan.

$$(C_6H_5)_3P + 2HCl \longrightarrow (C_6H_5)_3P\cdot 2HCl \quad (I)$$

$$\text{Ppt-L-Trp-OMe} + I \longrightarrow \text{Ppt-Cl} + \text{HCl}\cdot\text{H-L-Trp-OMe} + (C_6H_5)_3P$$

Ppt-L-tryptophan was prepared as follows. Ppt-Cl(126 g, 0.5 mol) was added to a solution of L-tryptophan(102 g, 0.5 mol) in 2*N* NaOH solution(250 ml). The mixture was stirred vigorously to start the reaction. After exothermic reaction had started 2*N* NaOH solution(250 ml) was added in a period of 10 min. Reaction

mixture was diluted with water(2 l) and extracted three times with each 300 ml of ethyl acetate. Aqueous layer was acidified by citric acid to pH 4 and extracted with ethyl acetate(1 + 0.3 l). Combined extracts were washed four times with 100 ml each of saturated $NaHCO_3$ solution and NaCl solution and dried over anhydrous Na_2SO_4. After removal of Na_2SO_4 dicyclohexylamine(100 ml, 0.5 mol) was added to afford Ppt-L-Trp-OH·DCHA which was collected by filtration and washed with ethyl acetate and ether. Yield 252.6 g(84 %), mp 187-191°C, $[\alpha]_D^{25}$ +7.5°(c 1, EtOH). Free acid was obtained quantitatively by removing DCHA by 5 % aq. citric acid solution. After recrystallization from EtOH it melts at 76-86°C. $[\alpha]_D^{25}$ -18.7°(c 1, EtOH).

Solid phase synthesis was performed as follows. Ppt-glycine benzyl ester resin(polystyrene-1%divinylbenzene, Gly content 0.38 meq/g) was placed in the reaction vessel of the Beckman model 990 peptide synthesizer. Ppt group was removed by treating twice with 2*N* HCl in methylene chloride containing 1*M* triphenylphosphine at 30°C for 30 min. After neutralization with 10 % triethylamine in chloroform Ppt-L-Trp-OH was coupled by the oxidation-reduction condensation method with use of tri(*p*-anisyl)phosphine[5] and 2,2'-dipyridyl disulfide. Deprotection of Ppt-L-Trp-Gly-resin was achieved without accompanying coloration by the same way as above. After the coupling of Ppt-L-Ala-OH protected tripeptide was cleaved from the resin by transesterification to afford Ppt-L-Ala-L-Trp-Gly-OMe in 60 % yield as white crystals, mp 171-173°C, $[\alpha]_D^{25}$ -32.5°(c 1, EtOH)

By the same procedures Ppt-L-Trp-L-Trp-L-Trp-OMe was synthesized in 68 % yield as white amorphous powder, $[\alpha]_D^{25}$ -62.5°(c 1, EtOH), starting from Ppt-L-tryptophan benzyl ester resin(Trp content 0.45 meq/g).

Since Ppt-amino acids are quite easily available, this new method would become a convenient way for the synthesis of tryptophan containing peptides. Further investigations are now in progress.

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